

Engineering—big issues and little issues

THE Finniston Committee to enquire into the engineering profession is just about to get underway. The committee will be able to investigate pretty well whatever it wishes under the general headings of the needs of industry, education and training, the institutions, and registration and licensing. Many hope that this committee will be the first step along the road to affording engineers their rightful status, whatever that may mean, and that a more prestigious profession will attract and retain better talent, thereby contributing to a revival in British industry.

There is no doubt that at present the engineering profession is not a remarkably well-paid one, that its institutions are in a state of considerable disarray, and that it suffers from association with the perennially bad news that is put about concerning British industry and industrial relations. As a result of all this people are hardly falling over each other in an attempt to become engineers.

Take, for instance, the institutions. Undeniably an engineer is not created the moment he (or very occasionally she) emerges from higher education, and a period of development needs to be watched over by some form of peer review, for which the institutions serve admirably. And for a rather small number of engineers the institutions serve a continuing function of education. But to meet these needs there are 15 institutions, each with its own traditions, bureaucracy and fine distinctions (as to, for instance, what a technician is and whether a technician can be a member). And now there is another layer, the Council of Engineering Institutions, born in considerable travail and watched very carefully by the individual institutions. It would be easy for the Finniston Committee to spend much of its time sorting out these institutional problems of engineering; what is more, the institutions on the whole favour some form of registration and licensing of engineers (this is not done at the present) and the committee could equally get bogged down in trying to produce a blueprint that was acceptable to all parties—when it is not at all clear that registering, licensing and thereby more effectively disciplining engineers is anything other than a sideshow generating bureaucracy to relatively little purpose.

Instead, there are two very clear and major problems that the committee should face. The first is that of recruitment into engineering, meaning not the choice

made on graduating as to which company to join, but the choice made in school, maybe as early as 14 or 15, to opt for engineering as opposed to the natural sciences. Arguably this decision is forced on students too young, with most universities singularly unhelpful in allowing students to defer their choice until they have experienced a year or two of higher education. But whatever time it is made, it seems remarkable that anyone selects engineering at all in the present educational environment. Few teachers have any industrial experience, and if they have had such experience, as likely as not they have been discouraged enough by it to turn to teaching instead. Few schools have a decent and open relationship with local industry—and where there is no local industry (as in the rural environment of most public schools) contact with industrial life may be nonexistent. Small wonder then that a life of engineering is seen by many young people as a poor alternative to a life of scientific research.

The second problem is that nobody really knows what the shape of British industry will be in ten or twenty years. In a completely cut-throat environment, many industries would shed vast numbers of workers, opting for much more automation at severely reduced manning levels, while other industries, such as shipbuilding, steel-making, and car manufacture might capsize because of foreign competition. In a more protective world, the government, aware of electoral implications, will fear to grasp any of these nettles but will prop up ailing industries and stand in the way of mass redundancies in the name of social policy.

In these circumstances it would be valuable to have a chart of possible paths for steering industry through to what is bound to be a totally different world by the year 2000. Economics and politics are bound to have major roles, but the engineering profession has a central part to play. Thus the Finniston Committee could provide a valuable service to the profession and to the nation by taking a longer-term view than governments ever seem to be able to take. It could also try to establish some general view of where industry will be by the end of the century. Some will carp that this is beyond the committee's remit, but if the needs of industry for engineers is one of the terms of reference then presumably the very character of that industry is rather germane to such discussions. □



Against instant books

Stephen H. Schneider of the National Center for Atmospheric Research, Boulder, Colorado, explains why he feels that 'instant' books often do more to confuse than elucidate the scientific controversies they discuss.

TIME and again in recent years a chorus of social pundits has bemoaned the increasingly hectic pace of modern living. Among the shopping list of adverse side effects frequently cited are the break up of the cohesiveness of the family unit, the rise in feelings of societal alienation of many young people, the increase of environmental pollution, the rise of junk foods, and the alarming rate of stress-related disease. To this growing list we must add a contribution from the publishing industry: the 'instant book'.

For example, very soon after the harsh "Winter of 1977" in the United States, as it is often called, we have *The Weather Conspiracy: The Coming of the New Ice Age* (Ballantine, New York, 1977). It has many of the trappings of an instant book. Since its 'author' is "The Impact Team", a group of 18 non-weather experts calling themselves reporters, writers, researchers, and "back-up" (whatever that means) people, they had to turn elsewhere for scientific credibility. They chose the wrong people.

Space doesn't permit a detailed critique of the two CIA reports on climate, which are the basis for *The Weather Conspiracy* and are included as appendices, and upon which the book leans so heavily for what it calls "true facts". I must, however, mention that Professor Reid Bryson of the University of Wisconsin, whom the CIA and the Impact Team cite as the expert predicting most of the coming climatic disasters, has publicly repudiated much of the CIA reports: and they quote him as a principal source of specific climatic predictions. Bryson objected for the simple reason that the predictions were specific—something which is beyond the state-of-the-art skills of climatologists. In fact, much of the CIA reports depend on the pre-1974 views of Bryson, and he has himself argued that new evidence has required him, as any good scientist, to revise and recast his views. In essence, I would characterise parts of the CIA reports that predict the climatic future as "Early Bryson extrapolated", and much of *The Weather Conspiracy* thus as "Early CIA extrapolated".

I must, however, confess nagging conflicts that bother me in using *The Weather Conspiracy* as a butt:

- it includes an impressive amount of material on climate, even if there is little cohesive thinking to link it together; and I don't want to take the purist role and discourage all mass market attempts to "spread the word" about the very real dangers climatic issues do pose for society merely because such polarisations simplify complex issues;
- many of the Impact Team's proposed solutions to these dangers, that is, food reserves, weather control treaties, energy conservation, and so on, while not new to those who follow the issues, are plausible and need widespread exposure—something a mass market book can do well;
- most importantly, as one whose own book, *The Genesis Strategy: Climate and Global Survival* (Plenum, 1976 and Delta, 1977), is itself an attempt to raise public consciousness about many of the issues repeated in *The Weather Conspiracy*, I am keenly aware of possible scepticism some might express about one author's seemingly pejorative treatment of a subsequent competitive book. The best that I can do to dispel any such possible suspicion is to state clearly why I believe a "pot boiler" like *The Weather Conspiracy* could really retard the efforts of those who seek to persuade society to anticipate and then hedge against the possibility of future climate-induced catastrophe—a goal that seems common to me and the Impact Team.

Commendably, *The Weather Conspiracy* does bore deeply into many of the issues of future climatic warmings and coolings, but instead of pointing out that either scenario for climatic change could be troublesome since much of human activity, particularly agricultural, is tuned to the present climate, it insists on maintaining the shock effect of the dramatic (the subtitle reads, "The Coming of the New Ice Age") rather than the reality of the discipline; we just don't know enough to chose definitely at this stage whether we are in for warming or cooling—or when. Nor, is *The Weather Conspiracy* alone in choosing sides in a scientific debate which is just not resolvable with present knowledge. Two other recent popular books by non-meteorologists give away their opposing advocacies in their titles: *Hot House Earth* versus *The Cooling*.

The damage to the authors' common cause of action on public policy from all three books is that they have been discredited publicly by many in the scientific community as sensationalist and technically inaccurate. Thus, in the confusing banter among experts—some pushing cooling, some pushing warming—the public and their elected officials usually shrug and say, "Let the scientists study more until they are sure what will happen".

That is precisely one of the greatest inadequacies that governmental institutions exhibit with regard to scientific controversies. They often confuse debates among scientists as a justification for a 'wait and see' attitude on policy considerations. Unfortunately, many controversial, unresolved scientific components of public policy issues, for example, the danger of nuclear power plants, the banning of saccharin or the landing rights of Concorde in New York, are not resolvable before decisions have to be made.

What policy makers need, therefore, is a realistic assessment of what is and isn't known about the science of problems like climatic change, along with some estimates of the vulnerability of different segments of society to a variety of plausible climatic scenarios; and also, some estimate of how long it might take the scientific community to reduce the large uncertainty that exists over the alternative projections of the future.

One hopes we do not need overstated scientific certainty to scare the system into action, for no doubt as soon as one group overstates the strength of scientific evidence to advocate a policy change, someone else advocating an opposing policy will be quick to point out the omissions or errors in the technical evidence, and will challenge the credibility of the original advocate's views—especially their policy options. The result is usually a delay in action, not a speed-up, for the added confusion slows up the process.

If accurate information is a key for society to survive the increasing complexity of the technological props that support its existence, then we must also learn to deal with the bewildering uncertainties surrounding the safety and acceptability of these props, and be willing to make value judgements as to whether we should hedge against the most plausible catastrophes that present knowledge can estimate. If this kind of common sense planning for insurance against plausible nasty surprises in the future can only follow from overstated cases shrieked out of instant books or from television news programmes; if we are unwilling to put in the time to follow in some detail just what science does and does not know about the range of potential technological crises, we will fall into crisis after crisis, under-reacting in advance and over-reacting afterwards.

As I have chosen *The Weather Conspiracy* as a point of departure to argue how books should not treat scientific controversies, let me return to it. Instead of meeting its page one stated purpose: "to inform the public of the true facts about a topic often clouded by fiction, superstition, and alarmist misrepresentation", *The Weather Conspiracy* leads the pack in clouding up further precisely what it is intended to clear. □

Japan's national R & D programme

John H. Douglas examines the progress of government-sponsored large-scale projects in Japan



A constructive way of using waste: garbage crushed into blocks for use in the building industry.

IF the continued economic success of "Japan Inc." still puzzles the average Westerner, the role played by research and development in fostering the country's industrial achievements remains even more of a mystery. Yet the conduct of research, like that of business, often reflects a typically Japanese character, and examination of research priorities reveals much about Japan's plans for the future.

The outstanding characteristic of the government-business relationship in Japan's "guided capitalism" is a continual search for consensus. When all parties can agree on a course of action, thorough commitment to massive projects can be secured and planning for a very long time-scale is facilitated. Similarly, the strength of Japanese research and development lies in the ability of government, universities and industry to commit themselves to joint effort toward long-term goals.

Not surprisingly, such research by mutual consent is usually limited to pursuing narrowly defined, immediately applicable ends. Inherently the system lends itself to adapting, rather than originating, new technologies, although this pattern may be changing. There are also structural limitations: nearly three-quarters of Japan's total R&D expenditures occur in the private sector (compared to less than half in Britain), which naturally leads to emphasis on applied, rather than basic, research.

John H. Douglas is a Fulbright research journalist in Tokyo.

Instead of directly sponsoring the majority of research, as in the West, Japan's government has more often played the role of midwife for consensus. But in the mid-1960s it became apparent that some major projects would require more direct government support. The Ministry of International Trade and Industry (MITI) was given the responsibility for sponsoring these large-scale projects, and in 1966 a National Research and Development Programme was established under the ministry's Agency of Industrial Science and Technology (AIST).

The partnership of government, industry and university was thus strengthened to be able to handle even more massive and long-range projects. To qualify for support under the programme, a proposed project had to have "urgent importance for upgrading national industrial standards, promoting efficient utilisation of natural resources, preventing industrial pollution, and so on".

Now, after a decade, some 14 projects have been launched under the programme. Two have been successfully completed: the development of an internationally competitive computer system and of a commercially viable desulphurisation process. One project, the development of a remote-controlled undersea oil drilling rig, was suspended in 1975 for reasons that MITI officials will still not discuss.

Current projects

A summary of the 11 remaining projects probably offers as clear a guide as any to the major areas of research and development that the Japanese believe should have the highest priority to serve as a base for future industrial and social development. The three outstanding exceptions to this statement include Japan's nuclear energy programme, the search for alternative energy sources called "Project Sunshine" and the country's emerging space programme, which are funded separately.

● **Jet engines:** In terms of anticipated total funding over the expected life of a project (see table below), the largest undertaking in the National Research and Development Programme is an effort to promote a technologically independent jet-engine industry. Specifically, the aim of this project is to produce prototypes of a turbofan jet engine particularly suited to Japan's domestic airlines, whose routes are

relatively short and require frequent stops. An engine with thrust in the 10 to 15 ton range is envisaged, with parts highly resistant to heat fatigue for frequent take-offs and landings.

Tests of a 6.5 ton thrust prototype are now being conducted in a British wind tunnel (there are none in Japan large enough for the task), and if these and later tests prove successful, a model of this engine may be mounted on an aeroplane within three years.

A related project, which may be funded separately and which has not yet been officially announced, is designed to help Japan's domestic airlines: a short take-off and landing (STOL) craft to be fashioned from the American C-1 military cargo plane. STOL capabilities will reportedly be incorporated by mounting jet engines on top of the plane's wings, so the plane could then carry about 125 passengers. The new project is expected to cost a total of about 17,000 million yen.

● **Pattern information processing:** Optical recognition of letters, numbers, *kana* (Japanese syllable characters) and *kanji* (Chinese ideographs) has now reportedly been achieved. MITI officials say the first practical application of the system can be expected within two years, probably in the patent office as an aid to processing applications.

This project also includes several parallel efforts including experiments with artificial intelligence and development of new electronic components. Active research is being sponsored in the fields of magnetic bubble devices, semiconductor lasers, holographic memories, very large scale circuit integration (megabit memories on a single chip) and microprocessor architecture for a variety of applications.

● **Magnetohydrodynamics:** MHD power generation, now the oldest continuing project in the programme, may turn out to be too expensive for even this sort of national effort. One senior MITI official says that MHD offers a "good opportunity for a joint project" with other nations, and he hints that active negotiations toward this end are already in progress.

Present Japanese targets call for testing a 100 kilowatt generator with a copper-iron magnet for 200 hours in 1980. Then, if these tests are successful, a 100 kilowatt generator with a superconducting magnet is planned for 1982.

● **Nuclear steelmaking:** In anticipation of the successful development of a multi-purpose high-temperature gas reactor (HTGR), funded separately, MITI is coordinating the efforts of more than a dozen major companies to produce the components that will be required by a steelmaking system based on the reactor. Some of the major goals of the first phase of this project include development of a 1.5 megawatt heat exchanger loop, alloys and insulation materials capable of handling the 1000 °C helium gas coming from the HTGR.

Parallel efforts include construction of a steam reforming test plant to make a reducing gas from light hydrocarbons, development of an apparatus for charging a shaft-type furnace under the new conditions, and completion of a conceptual design for the proposed system. If phase one is completed on schedule, by 1979, a nuclear steelmaking pilot plant is proposed, to begin operation as early as 1986.

● **Complex manufacturing:** Begun during the current fiscal year, this latest project aims at production of a single machine complex that can handle many successive manufacturing steps—such as grinding, milling, welding, forging and casting—at one location. Computer control and the latest laser technology will be incorporated.

● **New process for producing olefins:** Unsaturated hydrocarbons of the olefin series (general formula C_nH_{2n}) form the raw materials for many key petrochemical industries, and the Japanese fear that these industries will be threatened by anticipated shortages of imported naphtha, from which the country's olefin supply is presently produced. Since countries with domestic sources of natural gas or light oils are not so concerned with the naphtha problem, Japan has emerged as perhaps the world leader in developing a technology for producing olefins directly from crude oil, which is more likely to remain available.

During the first stage of the project, which ended in 1973, a test plant capable of treating five tons of crude oil per day was built. During the second stage, a 120 ton per day pilot plant is being constructed and experimental work is proceeding to see if olefins can also be produced from the residual oils left after vacuum refining of crude oil.

● **Resource and energy recovery.** Japan's extremely concentrated population (only about 20% of the country's land is flat) suffers acutely from problems that for many other countries are still only nuisances. The National Research and Development Programme has so far attacked four of these prob-

Japan's National Research and Development Programme		
Project	Anticipated Life	Lifetime Cost (10 ⁹ yen)*
Jet Engine	1971–1980	25.4
Pattern Recognition	1971–1980	25.0
MHD	1966–1982	18.4
Nuclear Steelmaking	1973–1979	12.3
Complex Manufacturing	1977–1983	12.0
Olefin	1975–1981	10.0
Resource Recovery	1973–1981	9.8
Traffic Control	1973–1978	7.3
Desalination	1969–1977	6.7
Electric Vehicles	1971–1977	5.7
(£1 ≈ 450 yen)		

lems: air pollution, urban waste, traffic congestion and water shortages.

To recover useful materials and energy from urban wastes, two 100 ton per day experimental treatment plants are now being developed. The feasibility of several of the technologies involved has already been demonstrated and the project is expected to be completed successfully within three or four years. Technologies now being considered for practical application include cryogenic shredding of wastes, magnetic and air-stream separation techniques, and internally heated fluidised-bed pyrolysis.

● **Traffic control:** To help drivers cope with some of the world's worst traffic congestion, what is probably the world's largest advanced traffic control experiment is now being conducted in a ward of southwestern Tokyo. In an area of about 30 square kilometers, traffic is being monitored by a highly automated control centre, which advises drivers of road conditions and routing options, through a variety of media.

Some 300 test vehicles have been equipped with a display panel that automatically tells a driver the fastest route to a predetermined destination. Another 1,000 vehicles have been provided with a simpler driving information unit that flashes such messages as "Road Construction Ahead". And three roadside display boards at key locations provide all drivers with notice of road conditions and recommended detours.

● **Water desalination:** For a country so notoriously damp and rainy (with more than twice the annual rainfall of Britain), Japan would seem to have little reason to give such high priority to a project for taking fresh water from the sea. But again because of the extreme concentration of people, water shortages are expected to begin in Tokyo and other major cities perhaps as early as 1980.

The MITI-sponsored desalination project, which ends this year, involved construction of a 100,000 m³/day test

plant using multi-stage flash evaporation (MSF) to produce fresh water. As a result of successful experiments at the plant, the Japanese now claim to lead the world in MSF technology, and private companies are exporting commercial desalination plants with capacities of 30,000 m³/day.

● **Electric vehicles:** In the other major project to be completed this year, five second-generation electric vehicles (two cars, two trucks and a bus) have been successfully designed, built and tested. MITI officials say the next step toward full commercialisation must be removal of legislative and economic disincentives and further improvement of accumulators for the vehicles.

During the fiscal year 1977, MITI's total expenditure for the National Research and Development Programme, including management cost, was 14,484 million yen (about £32 million). Thus, although the programme was designed to infuse government funds into areas of research that private companies might hesitate to enter alone, public expenditure remain relatively small. Commercial success in any one of the projects could conceivably bring to Japan enough foreign exchange to support the whole programme.

Rather than producing the "spin-offs" that result from open-ended research common in projects sponsored by Western governments, the MITI projects pull along a family of "spin-in" technologies. When, for example, a holographic memory or a new high-temperature alloy is finally developed, an eager market will be waiting. Theoretically, the disadvantage of even such long-range, loosely defined "applied" research is that it will not produce the sort of original breakthroughs that come haphazardly from basic research. So far, however, Japan has had little trouble importing these fundamental advances from abroad. As long as this flow of ideas remains adequate Japan's National Research and Development Programme will remain one of the country's biggest bargains. □

Sweden debates gene-splicing

FOR the first time, recombinant DNA research is being publicly debated in Sweden. Some voices are calling for broad understanding and judgement of the ethical, economic and political issues involved, while others are more concerned with the security aspects of a proposed P3 laboratory at Uppsala. At the same time, the Ministry of Education is setting up a one-man committee to see if existing Swedish laws are sufficient for the regulation of such research, or whether new legislation may be needed.

It sounds as though the country has suddenly woken up to find recombinant DNA research sitting on its doorstep. Not so. The previous government was approached about the issues as long ago as 1973, but did not respond. In 1975 Uppsala biologists applied for a P3 laboratory. In the spring of 1976 an eleven-man "Committee Concerning Research with Recombinant DNA" was set up under the auspices of the Natural Science Research Council, the Medical Research Council and the Swedish Cancer Society to ensure that such research was carried out in safety for both laboratory personnel and the public at large. The committee has decided that Sweden's risk classification system should be a combination of the British Williams' guidelines and the United States' NIH guidelines, adopting the stricter elements of each. Although the committee's mandate only covers state-supported research on recombinant DNA—and it must

approve all proposals for such research before they can begin—private industry has voluntarily agreed to follow the same procedure with its proposals.

According to the committee's chairman, Professor Peter Reichard of the Karolinska Institute, recombination of DNA within the same species (which requires only P1 facilities) is being done at the Karolinska Institute and the University of Uppsala, but so far no-one is doing any research needing P3 facilities. That part of the Uppsala group's work which has needed such a laboratory has been done so far at the Pasteur Institute in Paris. Private industry has not yet submitted any proposals to the committee.

Professor Lennart Philipson, who leads the Uppsala group, says he is tired of the controversy aroused by his proposed P3 laboratory. He complains that society's confidence in scientists is waning, and hopes that it will increase with public awareness of what the research involves. His new research programme, in which he will try to develop *Bacillus Subtilis* as an alternative host for recombinant DNA, will not need P3 facilities before 1979. The plans for converting part of an existing building into the new laboratory should be finished before Christmas. Critics of the scheme are demanding that they should be approved not only by the DNA committee, as was the original intention, but also by the local health authorities.

Wendy Barnaby

above the sea surface, shot another 30 m into the air (where some of it was dispersed and evaporated) and then rained down onto the sea. The pipe was capped after seven-and-a-half days. It was assumed that 40% of the oil had evaporated by then, leaving some 9,000 to 13,000 tons on the water. As its viscosity was low, it spread quickly; only 800 to 1,000 tons were recoverable by mechanical means. Chemical dispersants were assumed to increase the damage the oil would do to marine life, so they were applied very sparingly.

A week after the spill, the oil became granulated and later formed tar balls which, in June and July, were drifting over an area of 55,000 km² in concentrations averaging 2.5 mg m⁻² sea surface: a level considered to be heavy pollution. Close to the Bravo platform, oil-in-water emulsion under relatively freshly spilled oil was found to be in concentrations of up to about 300 mg/litre water: but further away, it was below levels which have caused acute sublethal effects on the more sensitive stages of fish development in laboratory tests.

At the time of the spill, biological development was in its early spring stage. In a few square nautical miles around and east of the platform, the degree of photosynthesis of the phytoplankton was significantly reduced: but otherwise its development seemed normal. There were few fish, fish eggs and yolk sacs in the area at the time of the spill and for a short time afterwards, and the number and distribution of fish seemed to be unaffected by the oil.

The institute sees several factors accounting for the lack of acute effects. The escaping oil was hot, and this, combined with the action of the wind and the fact that the oil spread quickly over the surface, meant that most of the volatile and toxic compounds were evaporated. What hydrocarbons remained in the sea were diluted by unstable water conditions. Excepting the area close to the platform, the resulting hydrocarbon concentrations in the water column were low.

When the "Tsesis" ran aground in the Stockholm archipelago on 26 October, between 1,500 and 2,000 tons of the 19,000 tons of medium-grade fuel oil she was carrying spilled out into the water. The coast guard managed to contain some of the oil with booms, but winds and currents carried a large part of it to an island and part of the mainland nearby. As the wind blew steadily in the same direction for a couple of weeks after the accident, the oil stayed banked up against the shores, emulsifying down to a depth of 2 to 5 metres.

According to preliminary results from tests carried out jointly by the University of Stockholm's Askoe

Geography affects oil spill damage

THERE have been two significant oil spills in Scandinavian waters this year: April's Ekofisk blow-out, and leakage from the Russian tanker "Tsesis" which grounded on an unmarked rock in the Stockholm archipelago in October. Although the Bravo blow-out dumped at least six times as much oil into the water as the "Tsesis" did, preliminary research suggests that marine life in the North Sea has not suffered as much as that in the archipelago. According to a Swedish expert, the reason for this is the different geographical factors involved in the two spills.

Preliminary findings published by the Institute of Marine Research at Bergen, Norway, describe the acute effects of the Bravo blow-out on fish and plankton as "small". An estimated 2,000 to 3,000 tons of oil at a temperature of more than 75 °C spouted daily out of an open production pipe 20 m



Meteosat, the European Space Agency's meteorological satellite, eventually reached geostationary orbit above the Gulf of Guinea on 7 December, after a series of false starts on the Cape Canaveral launchpad. The picture above is the first taken by the satellite at visible wavelengths, showing cloud cover above Africa and the Atlantic on 9 December 1977.

Laboratory and the Swedish Water and Air Pollution Research Laboratory, the effects so far have been relatively mild in the free water (the pelagic zone) but severe along the coast (the littoral zone). Because the accident happened in autumn, the water was cold and life processes were few and slow. This reduced the effects on both marine and bird life: less than 100 birds so far seem to have been killed. Preliminary estimates show that, within four days of the grounding, the mortality of the zoo-plankton within 400 m of the tanker had significantly increased, and about 35% of the zoo-plankton had either been touched by or ingested oil. What was dramatic, however, was the effect on the littoral zone's bladder wrack community. It was found to be almost completely deserted after the spill. Small crustaceans and molluscs either died or left the area. Laboratory studies have also indicated sublethal effects on mussels and snails.

Dr Olle Linden, who is leading the Swedish investigations, points out that the dilution factor is very important in determining the amount of damage done to marine life by oil spills. Severe ecological disasters are more likely to occur in shallow, enclosed coastal water than in the open sea, where the oil generally disperses quickly. The oil spilled from the 'Tsesis' was pinned by winds against the shore. In the end, it was the geography that made the difference.

Wendy Barnaby

Correction

The Food and Agriculture Organisation's (FAO) budget for the biennium 1978-79 will be \$211,350,000 not \$237,377,000 as indicated on page 553 of last week's issue.

UK Agricultural research is blooming

In contrast to the gloomy future foreseen for British science by many of its funding agencies, the UK Agricultural Research Council's (ARC) latest annual report for 1976-77, published last week, is almost cheerful. It reports a small growth for the Council's share of the DES Science Budget for 1976-77 and a period of stability in the Council's dealings with the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Agriculture and Fisheries for Scotland, the Council's main customers under the Rothschild customer/contractor principle.

The ARC's increased share of the Science Vote over the past two years (its grant for 1977-78 was 1.8% up in

More natural gas for Pakistan

PAKISTAN is the only country in the East that seems likely to cushion the energy crisis by exploiting its natural gas reserves. This has become possible because of two major discoveries of gas reserves within a matter of 12 months. According to Dr Shahzad Sadiq, Chairman of the Oil & Gas Development Corporation (OGDC), Pakistan is now in a position to export gas in liquid form or, as a gas, through overland pipelines. The western part of India is extremely gas-hungry and the possibility of piping gas to those regions is now being actively considered.

When last December a big natural gas reserve was discovered at Dhodak, in north Pakistan, the condensate oil in the reserve stole the limelight. The then Prime Minister, Mr Zulfikar Ali Bhutto, chose to dramatise it by bringing a bottle full of oil to the floor of the National Assembly (The Parliament), and waving it within inches of the face of the Leader of the Opposition. Pakistan, which watched the display on TV, was thrilled. Oil had been struck!

However, the Dhodak find is essentially a natural gas reserve with some 5 million million ft³ of gas. And with yet another big gas discovery only last month and again in the north (at Pirkoh in Baluchistan) the focus is now on natural gas exploitation. The Pirkoh gas reserves are estimated to match those of Dhodak.

The Chief Martial Law Administrator General Mohammad Zia-ul-haq, who at present heads the government, visited Pirkoh earlier this month to inaugurate the gas-field and formally underline the importance of the new find in the overall economy of the country. Pakistan suffers balance-of-payments difficulties which are

largely due to heavy imports of oil.

At Pirkoh, the OGDC Chairman told General Zia-ul-haq that an investment of \$250 million in the development of newly discovered oil and gas fields could give a return of \$230 million annually. This can be earned in oil import substitution and in export of gas and surplus petroleum products.

The gas fields at Pirkoh and Dhodak are not the largest in Pakistan. The biggest one is isolated at Sui in Baluchistan and has an estimated reserve of 10 million million ft³. It has been exploited for the last two decades. Pirkoh and Dhodak are graded next to Sui in reserve contents. The fourth largest gas field, at Mari in upper Sind, has also been exploited for some time; its estimated reserves are some 4 million million ft³. Total gas reserves in Pakistan are now estimated to reach 30 million million ft³.

The most significant impact of natural gas in Pakistan has been on agriculture. The four big fertiliser factories already in production (largely producing urea, also some ammonium nitrate and ammonium sulphate) entirely depend on the natural gas for the power and feedstock requirements. In addition, four more big fertiliser projects, based on natural gas, are under way. The green revolution that has almost doubled the average yield of wheat, the staple grain, and rice through the introduction of dwarf varieties within the last decade calls for plenty of fertilisers as its most vital input.

As a result of the two natural gas discoveries it should be feasible to set up more fertiliser and cement plants, apart from proposed exports to ease the balance of payment position.

Azim Kidwai

real terms on that for 1976-77), is attributed to a growing appreciation of the need for fundamental research in agricultural and related sciences by the Advisory Board for the Research Councils (ABRC). In particular, the ARC feels that it received sympathetic consideration when the ABRC allocated the extra money set aside for the research councils after the mini budget at the beginning of last month. Of the £4 million to be added to next year's Science Vote, the ARC will get £300,000. And of the £4 million to be spent on new capital work next year it will get £800,000.

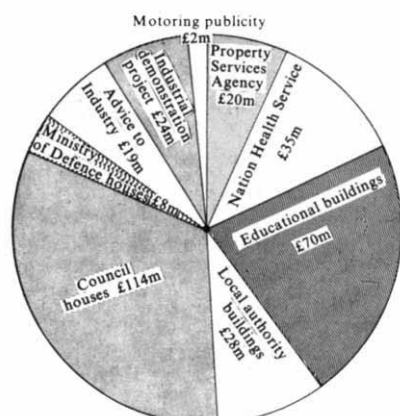
Sir William Henderson, ARC Chairman, attributes part of the Council's success in winning the

ABRC's sympathies to the work of the Priorities Working Party set up in June 1976 to select areas of basic agricultural research which could be most suitably funded from the DES Science Budget. Under the heading 'plants and soils' it chose crop variability, soil/root relationships and crop bioenergetics as priority topics for research. It has yet to select priorities in 'animals' and 'food' subjects. In line with the choice of priority topics, however, the £300,000 bonus is to be spent on setting up a programme of genetic manipulation in plants in several institutes.

The £800,000 which has been set aside for construction will most probably be spent on the improvement and the extension of existing institutes.

Judy Redfearn

Energy conservation comes of age



Expenditure on Energy Conservation
1978/79 - 1981/82

LAST week's announcement by UK Energy Secretary Mr Tony Benn of a £320 million package of government-sponsored energy conservation measures provided a firm indication that in Britain, as elsewhere in Western Europe, energy conservation has finally come of political age.

At meetings held last month by the Council of Europe in Strasbourg and the European Economic Commission in Brussels, speaker after speaker came to the platform demanding action on conservation with an enthusiasm unknown even six months ago. After the Brussels meeting, Herr Guido Brunner, EEC commissioner for energy, announced that the commission was likely to set up a new directorate concerned with energy conservation. And the German government has since announced a conservation package, including doubling the tax on home heating oil.

The largest part of the British package, which is to be spread over a period of four years, is the £28.5 million a year which is to be spent on providing loft insulation and draught-proofing measures for two million council houses. There will also be considerable sums of money allocated to government departments responsible for buildings.

As far as industry is concerned, a major expansion of information and advisory services will take place at a cost of £19 million over four years. Discussions are taking place with the motor industry about steps to reduce petrol consumption in new cars. And more than £20 million is being provided for an extended programme of demonstration projects, providing examples of the type of savings that can be made by individual companies.

Perhaps the most significant aspect of the British package is the financial investment that it implies in energy conservation measures. Last year a

Government White Paper stated that, while encouraging public sector bodies to finance such measures from their existing budgets, there was "no case for special financial assistance".

In last week's announcement, however, although Mr Benn repeated that programmes would be financed "as far as possible" from savings made elsewhere in existing programmes, over half of the initial £320 million budget is to be "new money". Only £93 million is being obtained from redirecting existing funds, the rest is to come from recycling the savings made from initial stages of the programme.

Another important indicator of government determination is the decision to establish a new Energy Conservation Division in the Department of Energy under Mr Bernard Ingham, for the past four years the department's director of information. The division will be responsible for developing and coordinating UK energy conservation policy.

Energy conservation has thus been clearly placed near the top of the department's agenda, receiving considerably more attention than two years ago when, in spite of the publicity surrounding the "Save It Campaign", the House of Commons Select Committee on Science and Technology dismissed the department's conservation efforts as "feeble".

The Select Committee did little to influence the department's strategy, which until now has relied primarily on example and propaganda. Since then, however, at least three factors seem to have contributed to the development of a more aggressive approach towards conservation.

The first of these has been President Carter's continuing determination to pass a wide-ranging energy bill through conference, providing the framework for a coordinated energy policy. A second factor has been the recent upturn in the British economy which, with the economic benefits of North Sea Oil beginning to be felt, have placed the government in a position to allocate financial resources to new programmes.

Perhaps most significant in political terms, however, was the summit meeting of head of state held in London in May. Energy conservation occupied a prominent position in the final communiqué, which stressed the need for "strict conservation measures" to enable an energy market to function harmoniously.

Shortly after the summit meeting, Mr Benn set in motion a major exercise to examine the scope for energy saving across all sectors of the economy. In particular, he asked for quantified calculations of the savings which could be achieved for specific

levels of public expenditure to obtain a substantial reduction in the growth of energy consumption.

Two other aspects added to the packet's political acceptability. The extra work involved in carrying out the massive programme of insulation—a labour-intensive activity—can be claimed to contribute to the general problem of unemployment (even though local governments may balk at some of the administrative implications). And the short-term, quantifiable benefits of conservation measures provide more tangible—and, to governments of short-time, more useful—political capital than measures whose impact can only be seen over ten years or more.

There remain reservations. Some have suggested that the proposed investment in insulation is too small to obtain maximum effectiveness, and that the measures will prejudice a second bite at the cherry. Others have complained that the package applies only to public sector housing, with no comparable efforts in the private sector.

And, of course, there remains the problem of energy conservation in industry, the biggest consumer of energy accounting for 40% of total consumption. Here the government's strategy remains essentially propagandist; two days after Mr Benn's package, Mr Leslie Huckfield, Industry Under-Secretary, claimed that appropriate measures could save industry up to £370 million a year, and that although this would require a capital investment of £560 million, "with such an incentive for reducing costs, they should be an attractive investment".

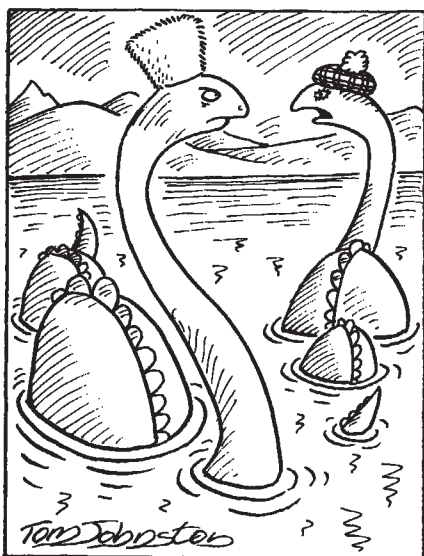
Industry itself remains sceptical, both about the Government's calculations with regard to what is practically (as opposed to theoretically) possible, and towards the claims of energy conservation as against other demands for capital investment, such as new equipment. An official at the Confederation of British Industry said last week that rather than offering financial incentives to industry for conservation measures, the government should reduce the general level of taxation, and let industrial management decide how to invest the extra money.

It remains to be seen whether the government will accept this *laissez faire* attitude. The Energy Commission's working paper emphasised that desired changes could not be left to price mechanisms alone, but that mandatory measures were needed to back up financial incentives. Possible measures could include new regulations about building design, petrol consumption of private motor cars, and so on. Mr Benn has demonstrated the size of the carrot; but we have yet to see the size of his stick.

David Dickson

Soviet New Year Diary

(to be taken with two vodkas and a pinch of salt)



"Better extinct than Red!"

Koskolteras Rhombopterix?

A Loch-Ness-type monster, 15 m long with a head 2 m long by 1 m wide has been reported in Lake Kos Kol, Kazakhstan. An unnamed commentator on Moscow radio observed that since several "extinct" species have recently been discovered to be still surviving, he considered it quite possible that "unknown creatures of the kind reported in both these lakes" might, indeed, exist. What he did not suggest, however, is any kind of joint Soviet-British (or Kazakh-Scottish) study project. This seems a strange omission; joint scientific research is a major feature in all discussions of mutual cooperation, and a possible monster investigation would enliven what too often become dull routine talks on oil drilling equipment and fertiliser plants. Indeed, should the two creatures prove to be of the same species, an interbreeding project might not be beyond the bounds of all possibility!

Bubble bath

Small boys on both sides of the space race have always been attracted to the profession of cosmonaut/astronaut—not least because of the obvious impossibility of taking a bath under conditions of weightlessness. Alas for childhood dreams—the days of a lick and a promise with a cologne-dampened washcloth are gone; Soviet technology has succeeded in equipping Salyut-6 with an experimental shower. Enclosed in a special shower cabinet, the cosmonaut is sluiced by a stream of water droplets borne by an airflow; the mixture of air and water being separated by a filter and recycled.

Un-cooking the books

According to geographer Arkadii Sopotsko, the geographical discoveries on the North West coast of the American continent, attributed to Captain James Cook, should rather be credited to Vitus Bering and Aleksei Chirikov 37 years earlier. The logs of the two explorers were long believed lost, largely because Imperial policy decreed that "Asia eastward cannot end", and evidence to the contrary constituted an important state secret. The missing logs have now come to light in the central Navy archives, and it now appears clear that when Bering, aboard the *Svyatoi Petr* (St Peter) reached the North American coast in July 1741 he mapped and named several features later recorded by Cook. These include the St Athengenes Ridge (now the Hayday Glacier), Cap St Mary (Cape Suckling), and islands of the Aleutian group which he named respectively for Sts Marcian, Stephen and Abraham.

To each according to his needs . . .

Aleksandr A. Bulgakov, Chairman of the USSR State Committee for Vocational Training recently announced that the text-book situation has greatly improved during the last year. There is now, he said, "a text-book per pupil in almost every [vocational training] establishment".

Up the Pole . . .

According to Aisultan Kalybaev, described by TASS as "a young Kazakh scientist", the terrestrial poles can move only by "tens of kilometres", and hence "the hypothesis that 500,000 years ago the North Pole was in the centre of the Pacific Ocean" must be false. This remarkable deduction, "based on the laws of mechanics" was apparently arrived at during research to provide a mechanical and mathematical basis for predicting earth-tremors in Kazakhstan.

Meanwhile a team from Leningrad and Yakutsk have established that the Siberian permafrost is retreating at the rate of 1 to 2 m "along the vertical line" (sic) per century. The melting is not due so much to climatic change as to an inflow of geothermal energy. Whatever the reason, it must be gratifying to the planners responsible for the development of the Soviet Far North to know that they have the backing of a natural process.

Whose was that baby . . . ?

The problem of the sailor, returning from a tour of duty, to be greeted by

an infant unbelievably forward or backward for his official age must be as old as Jason. Soviet seamen need no longer jump to the obvious conclusion, however; a recent broadcast on the seamen's service of Soviet radio reports the case of little Oleg Slonin, who at 14 months knew the entire Russian alphabet "except the hard and soft signs", and could recite 28 poems. The broadcast explained that "such a phenomenon is not very frequent, but is not now altogether rare. It is explained by the fact that, together with a physical acceleration, an intellectual acceleration takes place. Children nowadays have an early intellectual development."

In dock . . .

A new system for dockworkers, based on the study of "hourly, diurnal, and monthly" biorhythms is under test at the port of Odessa. In the service of productivity and safety, a computer is used to record for each man the "days notable for the maximum decrease of work capacity" and his "highest peak of inattention," according to his "biorhythm-activated schedule". The pattern obtained is used for planning work-schedules: a docker's days off will also be his "off-days".

Na zdorov'e

The Soviet anti-alcoholism campaign was inaugurated in the middle of the last 5-year plan—to the considerable disruption of targets for potable alcohol, which had suddenly to be replaced by beer and soft drinks. Although a considerable amount of the surplus vodka was unloaded onto the USA in return for pepsi-cola, the problem of hard drinking still remains pressing.

In addition to prosecution and/or compulsory committal for treatment, there is a widespread campaign of publicity and psychological pressure, including publication of crime and fatality statistics associated with the imbibing of alcohol, public rebuke of persistent offenders before their work-mates, and loss of privileges and bonuses. The latter sanctions are common Soviet practice in dealing with antisocial or "uncultured" behaviour—nevertheless, in this case there seems something wrong with the underlying psychology. A common factor in social drunkenness is that a family man living in a one-room apartment cannot conveniently invite his friends home, but must meet them in a bar. To relegate such a culprit to the end of the housing list hardly seems helpful.

Vera Rich

correspondence

Conserving uranium

SIR,—The exposition by John Davies (1 December, page 376) of the virtues of the CANDU reactor thorium cycle as a possible alternative to fast reactors does indeed cover matters "being discussed in undergraduate lectures twenty years ago". It does so with a splendid disregard for the present status of the technologies involved.

Recently the experimental fast reactor at Dounreay was shut down after 18 years of successful operation, its original exploratory task complete. An important part of that task was to demonstrate the docile behaviour and ease of control of fast reactors. The UK, France and the USSR are now operating prototype reactors in the 250–350 MW(e) output range. The maturity of the technology is apparent from the fact that the latter two countries have committed the construction of larger units of commercial significance.

The position on the CANDU-thorium fuel cycle is well set out in an authoritative manner in the evidence submitted by Atomic Energy of Canada Ltd (AECL) to the Ontario Royal Commission on Electric Power Planning which was published in April of this year. The section on 'Prospects for future CANDU fuel cycles' brings out the elementary fact that "Thorium is a fertile material but contains no fissile isotope". It is therefore proposed to use plutonium recycled from the existing uranium fuels to commence the cycle. Reprocessing of both uranium/plutonium and $^{232}\text{Th}/^{233}\text{U}$ fuels is therefore required—a more complex situation than arises with the fast reactor.

It is concluded by AECL that "the overall development and demonstration programme can be completed during the 1990s". This makes it quite clear that we are dealing with a technology which is not yet available even on a pilot scale, and which is unlikely to avoid the problems of MUF (material unaccounted for) and possible illicit diversion to weapons. Incidentally, freshly reprocessed ^{233}U is not automatically protected by gamma radiation, since this arises from ^{232}U daughter products, and takes about 10 days to build up to embarrassing levels requiring elaborate remote handling.

The AECL submission to the Royal Commission also discusses the use of accelerators under the heading of

'Electro-nuclear breeding'. Here it is concluded that "if the cost of uranium were to rise substantially, electro-nuclear breeding might be economically justified. In any event such systems will not be required until the advanced fuel cycles are fully established so there is ample time for their orderly development". The quotation places the Chalk River work cited by John Davies in its proper perspective.

In Canada, where there is a substantial investment in manufacturing facilities for CANDU reactors (including the heavy water production plants) the progress to the thorium cycle appears naturally as a logical step. At the expense of an initial increase in the rate of usage of uranium a long term benefit can be obtained. In my view it would not be sensible for this country to follow the same course when, with our starting point, (that is with fast reactor technology available) we are in a position to obtain a much larger energy output from the available stocks of uranium. The thorium reserves would of course also be burnable in fast reactors at a much later date if necessary.

D. HICKS

*Risley Nuclear Power Development
Establishment, UK*

Censuring repressive regimes

SIR,—The letter from Dr Peto and Professor Doll (1 December, page 384) asks several important questions, especially:

- how can busy scientists obtain a dispassionate assessment of alleged oppressive conditions in countries other than their own?
- What can they do to mitigate such oppression?
- In particular, is it useful to boycott scientific conferences in such a country?

These were just the questions which we examined in our report *Scholarly Freedom and Human Rights*, published earlier this year, in association with the British Institute of Human Rights, by Barry Rose. We concluded that the recent development of international human rights law had, for the first time in human history, pro-

vided a standard frame of reference, internationally agreed, against which the conduct of public authorities in different countries could be objectively measured. We recommended that there should be set up an independent clearing-house which would collect and evaluate information about such conduct in order that scientists could receive objective and impartial assessments on which they could rely, and we suggested the International Council of Scientific Unions and the International Commission of Jurists, jointly, as suitable bodies to undertake that task.

The ICJ has indicated its willingness in principle to take on its part of this function; the ICSU has not yet completed its examination of the proposal. Meanwhile, Peto and Doll were wise to consult Amnesty International: their sources of information are excellent, and their reports command world-wide respect.

There remains the question of the usefulness of boycotts. So far as I know, there is no evidence that public protest, or an effective boycott, have ever been counter-productive. There is some evidence that, on some occasions, they have influenced oppressive regimes for the better (the case of Dr Mikhail Shtern is a recent example). But in the absence of a full understanding of how the internal affairs of oppressive regimes are conducted, or of controlled experiments with matching samples, we can never know for sure.

The best procedure—at least in my view—would be for reputable international scientific bodies to agree in advance not to hold conferences and congresses in countries whose regimes have been clearly and impartially shown to pursue a consistent policy of oppression towards scientists, scholars and other non-violent citizens. There cannot be many countries which could survive such isolation for long.

This is a subject which has caused many scientists many personal and collective problems in recent years. We hope that the study of the underlying principles which is set out in our report can make its contribution to clarifying those problems, and indicate the directions in which their solution may be found.

PAUL SIEGHART

*Council for Science and Society.
London, UK*

Catastrophe theory

SIR,—It is not my purpose here to discuss in detail the criticisms of catastrophe theory contained in the review article by H. Sussman and R. Zahler (27 October, page 759). I would like to refer the interested reader, however, to a forthcoming article of mine entitled 'Mathématique et théorisation scientifique' to appear in *Scientia*. I would also like to point out a misquotation by the authors. The classification theorem for the "Cusp catastrophe", erroneously quoted as "Thom's theorem", is in this specific case due to H. Whitney (Mapping of the plane into the plane, *Ann. of Math.* 2, 62, pp. 374-410 (1955)).

RENE THOM

Institut des Hautes Études Scientifiques, France

SIR,—I would like to make two criticisms concerning Zahler and Sussman's recent review article on the applications of catastrophe theory (27 October, page 759). The first is that an editorial policy which sanctions the publication of polemical and emotive articles has no place in a scientific journal. In my view, the tone of Zahler and Sussman's comments goes beyond the lively discussions which we all welcome. Despite its many successes, there have been incorrect examples and applications of Thom's theory and extravagant claims for it. These should be assessed carefully and any errors rebutted scientifically. Zahler and Sussman's excessive and misleading criticisms do not help in the proper evaluation of the usefulness of Thom's theory and can only encourage a polarisation of opinion.

My second criticism is of the poor standard of argument and exposition in the paper and some comments on the first two pages follow. First Zahler and Sussman state that the record of legitimate uses of Thom's theory in physics and engineering is poor. But the theory has been applied very successfully in engineering^{2,3} and optics⁴.

Next, Zahler and Sussman appear to have missed the real weakness in the claim by Kozak and Benham⁵ and as a result much of their criticism is irrelevant. Thom's theory applies to processes governed by a potential function and with at most five controls, and which no matter how slowly the controls cross a threshold, pass from one equilibrium state to another through transitional states which are not in equilibrium. This passage between equilibrium states is continuous and often, though by no means always, very fast, particularly in mechanical cases, but never instantaneous. The discontinuities or 'jumps' arise if only equilibrium states are measured. Thom's theory does not apply if, when the controls are varied slowly enough, the process remains in equilibrium

throughout the transition. Consequently it is quite mistaken to suggest that the theory offers the cusp catastrophe as 'an inevitable, universal paradigm' for any system which exhibits sudden changes associated with two control parameters. Indeed because the process of collagen denaturation described by Kozak and Benham is of this kind^{6,7}, Thom's theory does not apply and one cannot expect collagen denaturation to conform to the cusp or any other catastrophe. The arguments based on the van't Hoff equation which Zahler and Sussman make in the section aptly headed 'Confusion about continuity' are quite irrelevant since the equation holds only under equilibrium conditions.

They go on to assert that 'most biological situations which catastrophe theory tries to model' [sic] are 'inherently continuous'. This is certainly true in the sense that the transition from one state to another, no matter how sudden, is still continuous at least above the quantum level, even if the process is not in equilibrium during the transition. It is not true if by inherently continuous Zahler and Sussman mean (as I think the context indicates that they do) that the transition states of such processes are equilibrium states. Genetic assimilation and quantum evolution are two important biological processes which involve transition states which are not in equilibrium. Moreover the concept of fitness and the widely used selection landscape of Simpson and Wright⁸ imply that evolution commonly proceeds from one maximally fit form to another through intermediate forms which are not maximally fit, that is not in equilibrium with their environment.

In fact, contrary to Zahler and Sussman's assertions on page 762 about my work, the notions of fitness and selection landscape permit Thom's theory to be incorporated naturally into the analysis of the adaptive response of populations subject to natural selection in slowly varying environments. This has been done for quantum evolution⁹, genetic assimilation¹⁰ and allopatric speciation¹¹. Two of these papers contain quantitative predictions for the response of the phenotype, although it is true that the imperfection of the fossil record and the variability in populations present great practical difficulties. However, these are problems which are common in applying mathematics to biology and are not peculiar to catastrophe theory.

Similarly in his work, Zeeman assumes a gradient system (a common enough practice in biology) so that Thom's theory can be applied. Moreover he makes many quantitative predictions which depend on the properties of the cusp catastrophe. There are a number made in 'Primary and secondary waves in developmental biology'¹², cited by Zahler and Sussman, particularly in §9

and §15. It is a limitation of the theory that some of these predictions can only be made to first order, as Zeeman makes clear in the second sentence of §9. Incidentally Zahler and Sussman's remarks about Zeno's paradox suggest that they have not appreciated this nor that at its vertex the cusp is flat. These predictions, in contrast to Zahler and Sussman's assertions, are not contained in the data, not independent of the theory and not just wrong. They need, as Zeeman suggests explicitly, to be tested experimentally and Zahler and Sussman's description of these predictions as 'purely unverified hopes' is a gross misrepresentation of what Zeeman has said, as reference to the paper just cited will show.

Zahler and Sussman do not make it clear that in spite of the underlying continuity, many important biological phenomena, such as somites, boundaries between different tissues, etc., are discrete and discontinuous. This provided a considerable stimulus to Thom's thinking and it is no accident that his theory offers a possible way of handling such phenomena mathematically. The most profitable procedure is to test the predictions which follow from applications of the theory and to see whether its geometrical framework gives useful insights.

A general comment is that the accomplishments or otherwise of the theory can be better assessed if a clear distinction is made between an example and an application, and between an explanation and a description.

MAURICE DODSON

University of York, UK

1. Zahler, R. S. & Sussman, H. J. *Nature* 269, 759 (1977).
2. Thompson, J. M. T. *Z.A.M.P.* 26, 581 (1975).
3. Thompson, J. M. T. *Nature* 254, 392 (1975).
4. Berry, M. V. & Nye, J. F. *Nature* 267, 34 (1977).
5. Kozak, J. J. & Benham, C. J. *Proc. Natn. Acad. Sci., U.S.A.* 71, 1977 (1974).
6. von Hippel, P. H. & Kwok-Ying Wong *Biochem.* 1, 664 (1962).
7. von Hippel, P. H. & Kwok-Ying Wong *Biochem.* 2, 1387 (1963).
8. Simpson, G. G. *The Major Features of Evolution* Columbia University Press, 1953.
9. Dodson, M. M. *Evolut. Theory* 1, 107 (1975).
10. Dodson, M. M. *Math. Biosc.* 28, 264 (1976).
11. Dodson, M. M. & Hallam, A. *Amer. Nat.* 111, 415 (1977).
12. Zeeman, E. C. in *Lectures on Mathematics in the Life Sciences* 7, 69 (1974).

Colour genes

SIR,—Our ignorance of the genetic basis of skin colour is not as profound as implied in Dr Bowne's letter (13 October, page 556). Nor do we have to assume the existence of unknown genes closely linked to colour genes in order to explain other traits that might be associated with skin colour, for there is evidence suggesting that the vast majority of pigmentation loci are probably pleiotropic (Deol, M. S. *Ann. Hum. Genet.*, 38, 501 (1975)).

Dr Bowne's views on intelligence are, of course, unexceptionable.

Yours faithfully,

M. S. DEOL

University College, London

news and views

Cell culture studies provide new information on tumour promoters

from I. Bernard Weinstein and Michael Wigler

MANY factors influence tumour incidence. Among these are the chemical carcinogens which, as a class, may act as mutagens. Tumour promoters are factors of an entirely different nature. They can be defined as agents which increase tumour incidence when administered after a suboptimal dose of carcinogen, but which are not in themselves carcinogenic. Recently, reports on the effects in cell culture of the most potent known promoting agents, the phorbol diesters, have raised expectations that an understanding of the action of these compounds *in vivo* is near.

The existence of tumour promoters was most clearly demonstrated more than 30 years ago by Berenblum and others who found that an oil extracted from the seed of *Croton tiglium* L. dramatically enhanced the incidence of skin tumours in mice pretreated with carcinogens. Croton oil was not by itself tumorigenic. The exposure of skin to carcinogens, was called 'initiation' and the subsequent step, the repeated application of croton oil or other agent, which elicited the growth of tumours from initiated cells, was called 'promotion'. Early work in this field established that the initiated state is extremely stable, extending perhaps for the lifetime of the animal. In fact, mice can be initiated with carcinogens *in utero* and, at some time after birth, tumours elicited by topical application of promoters (Goerttler & Lochrke *Virchows Arch.* **A372**, 29; 1976). Promotion generally leads to the development of benign papillomas; prolonged exposure results in the appearance of carcinomas. In 1968, the active principles of croton oil were isolated and their structures elucidated (Hecker *Cancer Res.* **28**, 2338; 1968; Van Duuren *Prog. exp. Tumour Res.* **11**, 31; 1969). They are fatty acid diesters of a tetracyclic plant diterpene alcohol, phorbol. Macrocyclic plant diterpene

esters of related structure, some of which are active as tumour promoters, are widespread in the plant kingdom. Other compounds and crude extracts, notably phenols, anthralin and extracts of tobacco tar, have tumour-promoting activity when tested on mouse skin, but no agents studied so far approach the phorbol esters in potency.

Recent observations on the action of the phorbol esters *in vitro* can be subsumed under two principles. The first is that nanomolar concentrations of tumour-promoting phorbol esters (but not their inactive analogues) induce changes in cultured cells which resemble those seen on transformation with either chemical carcinogens or tumour viruses, and further enhance the expression of these transformation-specific phenotypic features in already transformed cells. In chicken embryo fibroblasts (CEF), phorbol esters alter cellular morphology (Driedger & Blumbers *Cancer Res.* **37**, 3257; 1977), increase plasminogen activator synthesis (Wigler & Weinstein *Nature* **259**, 232; 1976), alter the composition of glycopeptides obtained from cell membranes (Weinstein *et al.*, in *Origins of Human Cancer*, Cold Spring Harbor Symp. quant. Biol., in the press), increase deoxyglucose uptake (Driedger & Blumberg *op. cit.*), and cause loss of LETS, the large external transformation sensitive protein (Blumberg *et al.* *Nature* **264**, 446; 1976). Similar changes are also seen when CEF are transformed by Rous sarcoma virus (RSV). The transformation mimetic effects of the phorbol esters are not all secondary to growth stimulation since they occur in CEF under conditions where growth stimulation does not occur. Phorbol esters enhance the expression of transformation-associated properties in transformed cells. The clearest example of this is the combined effect of the Rous sarcoma genome and phorbol esters on plasminogen activator production in CEF. Each factor alone increases the synthesis of the enzyme. Together they have a multiplicative effect (Weinstein *et al. op. cit.*). Synergistic action is also observed in alterations of the mem-

brane glycopeptides of CEF (Weinstein *et al. op. cit.*). O'Brien and Diamond have reported similar findings with respect to phorbol ester-induced ornithine decarboxylase levels in chemically transformed and normal hamster cells (*Symposium on Mechanism of Tumour Promotion and Cocarcinogenesis* Gatlinburg, 1977, in the press). If phorbol esters can enhance the expression of transformed properties *in vitro*, perhaps they can do so *in vivo* and thus provide a preferential growth advantage to latent tumour cells.

The second principle is that the phorbol esters reversibly inhibit terminal differentiation. This effect was noted independently in three laboratories using two systems: chicken embryo myoblasts undergoing myogenesis (Cohen *et al.* *Nature* **266**, 538; 1977), and Friend erythroleukaemia cells (FEC) undergoing either spontaneous or induced erythroid differentiation (Rovera *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 2894; 1977; Yamasaki *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 3451; 1977). These initial observations have been extended to the differentiation of 3T3 cells to lipocytes (Diamond *et al.* *Nature* **269**, 247; 1977), chondrogenesis of chicken embryo chondroblasts (Pacifci & Holtzer *Am. J. Anat.* **150**, 207; 1977), and differentiation of neuroblastoma cells in culture (Ishii *et al.* *Science*, in the press). These observations and their generalisation, if applicable to mouse skin, provide a seductively simple interpretation of initiation and promotion. The stem cells for epidermis are continually dividing, yet the tissue as a whole is in a state of balanced growth, and a stable stem cell pool size is maintained. This is possibly achieved by a regular asymmetric division of the stem cell: one daughter cell becoming a stem cell and one daughter cell terminally committed to differentiate, irreversibly losing its growth potential. If a potential tumour cell were restrained to the stem cell mode of division, it could not increase its proportion in the stem cell pool. If, however, the stem cell division mode were interrupted by the action of a promoting agent, a potential tumour

I. Bernard Weinstein is in the Department of Medicine and Michael Wigler is in the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York.

cell could increase its proportion in the cell population and give rise to a tumour. Two cautions must be noted in the exercise of this hypothesis. First, phorbol esters greatly potentiate transformation by chemical carcinogens in cell culture systems (Lasne *et al.* *Nature* **247**, 490; 1974; Mondal *et al.* *Cancer Res.* **36**, 2254; 1976) where the stem cell concept may not be applicable. Second, phorbol esters have not been shown to inhibit terminal differentiation of mouse skin.

The two generalisations of recent

data on the action of phorbol esters in cell culture, that they mimic and enhance the tumour phenotype and that they inhibit terminal differentiation, are preliminary but provocative. The factors which determine tumour incidence, latency and rates of tumour progression are largely unknown. Studies on the cellular and molecular basis for the action of the phorbol esters and similar compounds may clarify these aspects of carcinogenesis and suggest novel approaches to the control of neoplasia. $\square$

Limits to similarity among coexisting competitors

from Henry S. Horn and Robert M. May

ABOUT 50 years ago, the theoretical work of Lotka and Volterra and the laboratory experiments of Gause, Park and others led to the formal enunciation of the 'competitive exclusion' principle: species that make their livings in identical ways cannot coexist. Although this principle may appear fundamental, it is not really very helpful. As stressed by Hutchinson, in his classic 'Homage to Santa Rosalia, or why are there so many kinds of animals?' (*Amer. Nat.* **93**, 145; 1959), the meaningful question is rather how similar can species be, yet persist together? What are the limits to similarity?

Most competitive situations involve many different ecological factors, which are too difficult to disentangle with present methods. But insight can be gained from those special situations where competitors sort themselves out

mainly along a single resource axis, such as food size or foraging place. Hutchinson catalogued many examples, drawn from both vertebrates and invertebrates, of sequences of competing species in which the average individuals in successive species have weight ratios around 2. This implies ratios of about the cube root of 2, or 1.3, between typical linear dimensions (for example, beak length) of successive species. Many other examples have subsequently been tabulated, particularly for birds, lizards and frogs, by MacArthur, Diamond, Cody, Schoener, Pianka, Toft and others. In an extensive study of guilds of birds on various West Indian islands, Faaborg (*Amer. Nat.* **111**, 903; 1977 and in the press) has shown that the weight ratio of roughly 2 holds for passerines, but that non-passerine sequences typically have smaller weight ratios.

Recent studies of the workings of the rule among congeneric sequences of invertebrates tend to be more equivocal. Uetz (*J. Anim. Ecol.* **46**, 531; 1977; see also Enders, *Environ. Entomol.* **5**, 1; 1976) has shown that part of the structure of his guild of 10 species of wandering spiders in an oak-maple woodland in the eastern United States conforms to the 1.3 ratio; some of the species with very similar sizes further subdivide their habitat by flourishing at different times in the season. Similar stories are told of carabid beetles and guilds of spittlebugs (by Southwood and Halkka, respectively, *Royal Entomological Society Symp. on Insect Faunal Diversity*, Imperial College, September 22–23, 1977). Again the 1.3 length ratio plays a part for those species that are very similar in other respects.

The magic 1.3 ratio is no newcomer to the biological literature. Dyar (*Psyche* **5**, 420; 1890) long ago noted that successive larval instars of many insects have weight ratios of 2, and linear ratios of 1.3. This provoked much discussion, which seems to have been forgotten, and even speculations that the underlying mechanism was a doubling of the number of cells between instars (later shown to be not generally the case; for a review, see Bodenheimer, *Q. Rev. Biol.* **8**, 92; 1933).

This empirical relationship is still not understood. When the size of food is considered, various lines of argument lead to the expectation that the average difference in food size between two species should not be appreciably less than the characteristic range of food sizes utilised by either species. This, however, does not explain why the typical intraspecific range of food items spans a weight ratio of around 2.

Before too much time is spent theorising about the Dyar–Hutchinson rule, it should be noted that it appears in many other contexts. In the conventional ensemble of recorders, the lengths of soprano, descant, treble, tenor, bass and great bass are in the ratios 1.2, 1.5, 1.3, 1.6, 1.4. For the consort of crumhorns, the heights of descant, treble, tenor and bass go as 1.2, 1.5, 1.4. The usual consort of viols have typical lengths of treble (61 cm), tenor (86 cm) and bass (110 cm) in the ratios 1.4, 1.3; the alto (70 cm) and viola d'amore (85 cm) are competitively excluded and are relegated to solo roles.

The four string instruments of the modern orchestra—violin, viola, cello, double bass—are grossly discrepant with the rule, having length ratios 1.2,

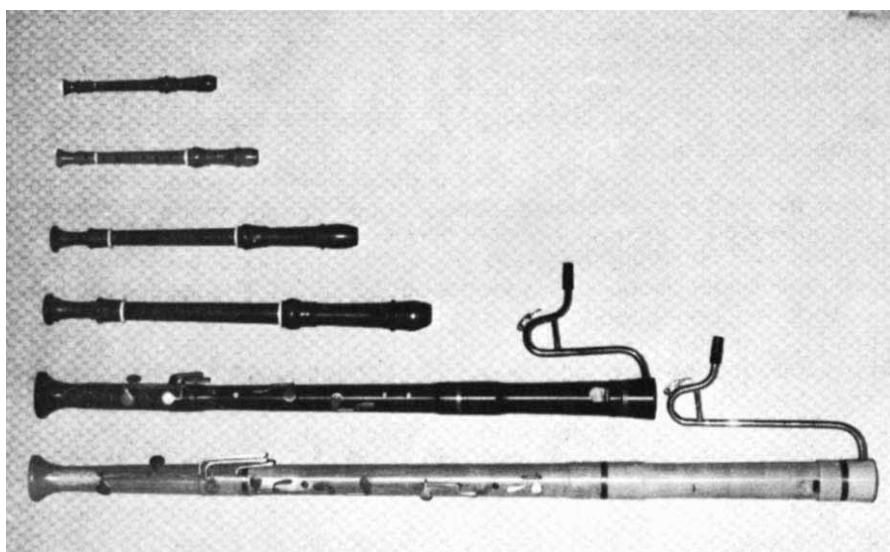


Fig. 1 The conventional ensemble of recorders, whose lengths roughly obey the '1 : 3 ratio rule'.

Henry S. Horn and Robert M. May are members of the Biology Department at Princeton University.

1.8, 1.7. This apparent counter-example is instructive. Taking advantage of acoustic experiments begun 40 years ago (by Saunders, a Harvard physicist), Carleen Hutchins has designed and built a 'new violin family'. This is a unique collection of eight stringed instruments, each of which has the general shape common to members of the conventional violin family. The new violin family has resided in London since it first arrived there in 1974, and it was recently the central attraction at a meeting of the Catgut Acoustical Society (see Darius, *New Scientist* **175**, 21 July; 1977). Darius writes 'Not since the consort of viols has there been an ensemble of acoustically matched stringed instruments . . . What distinguishes the new instruments is the rich, even tone quality . . . The ensemble of these new instruments particularly shines in contrapuntal writing'. For the family, the lengths of sopranino, descant, treble, alto, tenor, baritone, bass and contrabass are in the approximate ratios 1.2, 1.2, 1.3, 1.3, 1.3, 1.3, 1.3.

Ranging yet more widely, we note that the larval instars of children's tricycles and bicycles have typical wheel diameter ratios of 1.3, 1.2, 1.3, 1.2; the adult stages segregate their niches by differences in gear-shift rather than in wheel diameter. One manufacturer's set of five iron skillets gave size ratios of 1.2, 1.2, 1.2, 1.3.

In short, the Dyar-Hutchinson-Hutchins rule may well derive from generalities about assembling sets of tools, rather than from any biological peculiarities. □

Antimatter

from P. I. P. Kalmus

THE strong and electromagnetic interactions between two antiparticles should be the same as those between corresponding particles. Thus antinucleons should bind together to form antinuclei, and these could capture positrons to form antiatoms. This suggests the intriguing possibility that bulk antimatter might exist in the Universe. This would be exactly as stable as matter, provided that it was isolated. Searches for cosmic antimatter have given negative results, but have hitherto been limited to two methods. A new method of distinguishing between remote matter and antimatter has been proposed recently by J. G. Cramer and W. J. Braithwaite (*Phys. Rev. Lett.* **39**, 1104, 1977).

We would not be able to distinguish between the spectrum of an antistar

and an equivalent star, since the atomic energy levels would be identical and any photon reaching us is its own antiparticle. One way of testing the antimatter hypothesis would be the detection of annihilation when opposite regions collide. An electron and a positron at rest annihilate predominantly into two photons. These must be emitted in opposite directions in the centre of mass each with 0.511 MeV, the rest mass of the electron. The low energy proton-antiproton annihilation is mainly into more than two pions which are therefore not kinematically constrained to be monoenergetic. The decay of the neutral pion gives rise in its centre of mass to two monoenergetic photons of 67.5 MeV, but these would be greatly Doppler-smeared by the relativistic motion of the pion, and would give a fairly broad spectrum with a peak at around 100 MeV. This has not been observed in gamma ray astronomy, and neither has the distinctive 0.511 MeV line. However, H. Alfvén has claimed that this does not exclude the existence of antimatter. It is possible that annihilation would not occur with high efficiency because the tenuous regions of interstellar gas will come into contact first and the resultant pressure of annihilation radiation might keep the matter-antimatter regions apart. Moreover, this initial contact might well be between relativistic electrons and positrons which would not give the 0.511 MeV line.

Convincing evidence for cosmic antimatter would be its detection in the primary cosmic radiation. The detection of anticarbon or heavier antinuclei would indicate the existence of nucleosynthesis in antistars. Several groups have searched for such antiparticles including G. F. Smoot and colleagues who have flown a superconducting-magnet particle spectrometer on a balloon. No antiprotons or antinuclei have been found, the upper limit to their abundance being less than 10^{-4} of the corresponding nuclei. It seems unlikely therefore that significant amounts of antimatter exist in our Galaxy.

Fusion processes and nucleosynthesis in stars systematically convert protons into neutrons and release neutrinos. The corresponding reactions in antistars would release antineutrons and so in principle suggest a test for antimatter. However, as solar neutrinos are only just detectable by present techniques, neutrino astronomy of specific distant objects does not seem to be imminent.

The method proposed by Cramer and Braithwaite makes use of the violation of parity and of particle-antiparticle conjugation in the above weak interactions. These processes, in stars,

involve the emission of positrons or the capture of electrons. The positrons are preferentially in a right helicity state, that is, their spins are aligned along their direction of motion. If their emission is accompanied by inner bremsstrahlung or if they produce external bremsstrahlung when slowing down in matter, the helicity will be transferred to produce right-circularly polarised photons. Annihilation of the moving positrons by stationary electrons will cause the forward-going of the two photons, which carries most of the energy, to have the same helicity as the positron. Finally, internal bremsstrahlung which accompanies electron capture will also have a preferred right helicity.

The equivalent reactions in antistars would convert antiprotons into antineutrons and produce electrons or capture positrons. Following the above arguments, bremsstrahlung and annihilation will result in the emission of left-circularly polarised gamma rays.

The question arises as to whether such polarised radiation is actually observable. Normally the nuclear reactions take place in the core of the stars, and the radiation may be absorbed and re-emitted an enormous number of times before escaping from the surface. It seems most unlikely that significant traces of the original helicity will remain in starlight. However, a supernova, even one located in another galaxy offers the possibility of observing this helicity. This type of explosion is believed to occur when fusion in a massive star has terminated with the production of ^{56}Ni at the peak of the binding energy curve. The ^{56}Ni decays into ^{56}Co and then into ^{56}Fe by electron capture and positron decay. Assuming that the stellar envelope blows off during the supernova explosion, much of the released photon energy occurs at the surface. Also, the positrons are emitted in heavy medium, namely iron rather than hydrogen, which would enhance the probability of bremsstrahlung by a factor 676, the ratio of the squares of the nuclear charges.

The authors estimate the photon flux from supernova in which 0.14 solar masses of ^{56}Ni is released at a distance of 1 Mpc. In a period of 1 year starting 2 weeks after the initiation of the supernova is seen, a 1 m diameter polarimeter would receive about 3×10^5 photons from inner bremsstrahlung, having mean energy 0.2 MeV and 25% polarisation, and possibly a factor 10^2 to 10^3 more photons from external bremsstrahlung. The helicity of gamma rays has been determined by laboratory experiments by their differential scattering from polarised electrons, but polarimeters

for use in space, and preferably with higher analysing power, would have to be developed if gamma-ray astronomers hope to find antimatter by this method.

Manipulation of industrial microorganisms

from Juan-Francisco Martín

The fifth FEMS Symposium entitled Antibiotics and other Secondary Metabolites: Biosynthesis and Production was held on 14-16 September at the Ciba-Geigy Research Laboratories in Basel (Switzerland) under the auspices of the Federation of European Microbiological Societies.

WHY microbial strains used in industrial processes produce such large amounts of a particular metabolite in spite of the strict control of metabolism existing in most microorganisms, and how it is possible for scientists to improve the production of pharmaceuticals such as antibiotics, ergot alkaloids and so on, was the main subject of the symposium. One of the great advantages of this meeting was the participation of scientists from the academic world and industry both as speakers and in the audience. The limitation usually imposed by industrial secrecy was not so evident in this meeting and the flow of scientific knowledge in the lectures and discussions as well as in the corridors was quite free.

New approaches to the search for new metabolites of industrial importance were discussed in the first lecture by H. Zährner (Tübingen). Some of the new techniques he suggested such as 'the chemical test systems' where the search for totally new antibiotics is based on screening unusual metabolic products following feeding of the culture with fluor or sulphur radioactive label, were compared with more classical approaches in the search for new drugs such as the tests of antimicrobial and other biological activities or the use of cell free test systems for highly specific activities. It was the feeling of most participants that while the search for unique chemical structures may be useful, more rapid progress can be made using specific 'in vitro' assays to look for inhibitors of those enzymes

Juan-Francisco Martín is a Research Fellow at the Instituto de Microbiología Bioquímica (CSIS) and Assistant Professor at the Departamento de Microbiología, Facultad de Ciencias, Universidad de Salamanca.

Coupled transcription/translation

from Pamela Hamlyn

OOCYTES isolated from the ovary of the frog *Xenopus laevis* made their reputation as a valuable tool for molecular biology when they were used to settle disputes concerning the function of the poly(A) tail—the string of adenylic residues added post-transcriptionally to eukaryotic mRNA. Translation of mRNAs into protein in heterologous cell-free systems proceeds for a few hours at most, compared with several days when mRNA is injected into oocytes and so it is not surprising that only in the oocyte translation system was it shown conclusively that the poly(A) tail ensured the stability of mRNA (Huez *et al.* *Eur. J. Biochem.* **59**, 589; 1975).

Although the translation of mRNA into protein has proved to be reasonably amenable to study in cell-free systems the faithful transcription of RNA from DNA has been difficult to reproduce *in vitro*. It now seems that the use of oocytes will have an important role in elucidating the mechanism of transcription and other related problems of gene action, particularly its control.

Recently Gurdon, De Robertis and Partington (*Nature* **260**, 116; 1976) demonstrated that nuclei from human tissue culture cells continue to function (synthesize RNA) when injected into oocytes. Further work from the group showed that intact nuclei were not necessary, but that isolated DNA injected into the nucleus of oocytes could be transcribed into RNA (Mertz & Gurdon *Proc. natn. Acad. Sci. U.S.A.* **74**, 1502; 1977). Several DNAs were found to be effective, but most of the experiments were done with simian virus 40 DNA. It is important to be certain that the new RNA synthesised is transcribed from the SV40 DNA and is not new oocyte RNA transcribed in response to the foreign DNA. To achieve this the authors compared the RNA with that

transcribed when SV40 is growing in its normal host (monkey cells) and found that they were the same size and hybridised to the same region of the viral genome. The same group has now produced much better evidence, not only that the RNA is viral in origin, but also that it is being transcribed correctly. De Robertis and Mertz report (*Cell* **12**, 175; 1977) that they are able to detect specific viral proteins in the oocytes after injecting SV40 DNA into the nuclei. The extra proteins were detected by displaying the total cell protein using the 2-dimensional polyacrylamide gel electrophoresis separation method of O'Farrell. If an inhibitor of SV40 DNA transcription was injected together with the DNA the new proteins did not appear and, most convincing, if SV40 mutants, which code for smaller viral proteins in their normal host, are injected into oocytes the new proteins produced in the oocytes are correspondingly smaller. These results clearly establish that the viral DNA is correctly transcribed in oocytes.

The demonstration that the RNA can be translated into the expected protein is good evidence that the initial transcription was biologically significant—starting and stopping at the right place. It is exactly this kind of precision which has been difficult to achieve in *in vitro* cell-free systems and which is essential for detailed experiments on the control of gene action.

De Robertis and Mertz have also shown that *Drosophila* histone genes cloned in a plasmid have produced 'histone-like' protein in the oocyte coupled transcription translation system indicating that this technique will be of general application in the study of the control of genetic expression.

Pamela Hamlyn is at the MRC Laboratory of Molecular Biology, Cambridge.

which are known to be involved in the synthesis of essential microbial macromolecules or in animal cell transformation in cancer formation.

The denomination of the so-called 'secondary metabolites' was the subject of an interesting discussion. It was felt that although these compounds present some peculiarities (for example, their chemistry is unusual, they seem to have no essential role in the survival of the producer strains since the ability to produce them is easily lost by mutation, and their production is restricted to certain taxonomic groups), there are

no major biochemical differences in the biosynthesis of these metabolites to justify a separate grouping under the name of 'secondary metabolites'.

The new approaches to the biological and bioengineering aspects of fermentation development were presented by J. F. Martín (Salamanca) and M. Kuenzi (Basel) respectively. The general feeling was that we are on the threshold of great advances in the field of genetic and biochemical manipulation of industrial microorganisms, whereas new developments in bioengineering, such as programmed sub-

strate feeding and the development of new methods of estimation of fermentation parameters will occupy a second place in improving yields in antibiotic production. Martin discussed new advances in gene amplification using molecular cloning techniques, and alteration of the regulatory mechanisms controlling antibiotic production, namely: induction, carbon and nitrogen catabolite regulation, feedback regulation and phosphate (energy charge) regulation. A large number of studies including those presented at the meeting by Z. Vanek (Prague) on the phosphate regulation of tetracycline biosynthesis and H. Pape (Münster) on the carbon catabolite regulation of tylosin biosynthesis, led to the conclusion that deliberate genetic removal of regulatory mechanisms involved in antibiotic biosynthesis is a useful tool to increase antibiotic production in the immediate future. On the other hand, there was a lively polemic on genetic engineering as a tool for the amplification and transfer of genes coding for antibiotic synthesis. While some scientists considered feasible, and perhaps convenient, the possibility of transferring the capability to produce antibiotics to microorganisms other than the natural producer, others contended that it may not even be desirable, let alone feasible, bearing in mind that so little is known about the genes for antibiotic biosynthesis.

Recent progress in the area of biosynthesis of specific groups of antibiotics (tetracyclines, rifamycins and macrolides) were described by Vanek (Prague), G. Lancini (Milano) and H. Grisebach (Freiburg) respectively. Also, advances in the field of β -lactam antibiotics were discussed in detail. E. P. Abraham (Oxford) presented recent results from his laboratory and from others on the complexity of the stereochemistry of ring-closure in the formation of penicillins and cephalosporins. New genetic and regulatory approaches to the development of penicillin production were reported by C. Ball (Ulverston). J. Nuesch and his coworkers H. J. Treichler and M. Liersch (Basel) reported unpublished results of his group at Ciba-Geigy on the methionine biosynthetic pathway in *Cephalosporium acremonium* that provide insight into the precursor and regulatory effect of methionine stimulation of cephalosporin production. All these reports in the field of biosynthesis and regulation of β -lactam antibiotics together with the discovery of new β -lactam antibiotics and β -lactamase inhibitors produced by species of *Streptomyces* and *Nocardia* (the cephamycins, nocardicin, thienamycin, clavulanic acid) point to a renaissance of interest in this interesting group of antibiotics.

Secondary metabolites with no antibiotic activity such as the alkaloids were also considered. Advances on the chemistry, biochemistry and the cell fusion of producer *Claviceps purpurea* strains using protoplasts were reported by Groger (Halle), C. Spalla (Milano) and H. Kobel (Basel). New examples of the use of microorganisms in the specific biotransformations of chemicals and pharmaceuticals were described by K. Kieslich (Berlin) and H. Leuenberger (Basel). These once more illustrate the great possibilities, the advantages and the limitations, of enzymatically catalysed reactions for the modification, as well as the total synthesis of natural products. □

Nuclear quadrupole resonance spectroscopy

from J. A. S. Smith

The Fourth International Symposium on Nuclear Quadrupole Resonance Spectroscopy was held on 13–16 September, 1977 at the Takarazuka Hotel, near Osaka, Japan. The local Chairman was Professor H. Chihara of Osaka University.

THESE symposia review progress in a branch of radiofrequency spectroscopy very much concerned with structure and molecular motion in solids. There was an air of practicality about the proceedings of this year's conference, appropriate in the light of the ideas of the 8th century Japanese monk, Saicho, who considered that scholars should 'serve in such undertakings which benefit the nation.' The practicality appeared almost immediately in the description of a precise nuclear quadrupole resonance thermometer whose absolute accuracy was claimed to be ± 2 mK (A. Ohte, Yokagawa Electric Works).

The opening paper was given by E. L. Hahn (University of California, Berkeley), whose laboratory over the past 15 years has done much to stimulate the development of new instrumental techniques in radiofrequency spectroscopy; his paper was concerned with the double resonance detection of ^1H quadrupole resonance in solids, with the minimum possible degree of enrichment—in this case, 1%. Such a proposal would have been unthinkable some 10 years ago, and

illustrates the extent to which double resonance techniques have developed in recent years. In the following papers, the emphasis was very much on consolidation and application of these advances, from a triple resonance detection method for ^{17}O in natural abundance (R. Kado, Kyoto Sangyo) to detailed studies of doped alkali-halide crystals (T. Taki, Tokushima). Later papers on the instrumental side laid much stress on pulsed techniques and R. A. Marino (Hunter College, CUNY, New York) described a particularly elegant pulsed system with Fourier transform capabilities for ^{14}N quadrupole resonance detection, which is now being developed commercially. Together with the development of such instruments, the increasing use of relaxation measurements in chemical and physical problems was very obvious, with H. Chihara reporting on molecular motion in 1,2-dichloroethane, R. Hewitt (University of California, Riverside) on ^{121}Sb , ^{123}Sb relaxation in the metal, A. Colligiani (CNR, Pisa) on ^{14}N relaxation in benzonitriles, and Y. Abe (Tsukuba) on a careful investigation of structure and motion in hydrazine. Solid state effects were the subject of considerable interest with a survey by G. K. Semin (Institute of Organo-Element Compounds, Moscow) of the wide-ranging studies of electric field effects made by his research group in recent years, and a report by T. Kichi (Osaka) on intermolecular interactions in $\alpha\text{-ICl}$. Another current field of interest is the potential application to biological problems, a field as B. Lindman (Lund) showed where measurements of quadrupole relaxation times in solution have contributed much new information on ion binding in model membrane systems.

In inorganic applications, much new and important work was also presented. T. C. Waddington (University of Durham) reviewed recent work from his laboratory on the ICl_4^- and AuCl_4^- ions, which seem to show, in many of their salts, a kind of *trans* influence in their interionic interactions.

Several new Zeeman studies were reported, among which may be mentioned that of H. Negita (Hiroshima) on ^{81}Br quadrupole resonance in NaAl_2Br_7 , in which the bridging ^{81}Br frequencies were definitely assigned; in an entirely independent set of experiments, A. Weiss (Darmstadt) had also made the same assignment by $^{81}\text{Br}/^{27}\text{Al}$ double resonance (SEDOR) experiments, distinguishing between bridging and terminal signals by comparing their response to ^{27}Al irradiation. Fine structure of the ^{127}I resonance due to In-I spin-spin coupling was reported in In_2I_6 (K. Yamada, Hiroshima) and theoretical analysis suggests that the J -tensor is not axially symmetric. □

J. A. S. Smith is Professor in the Department of Chemistry, Queen Elizabeth College, London.

International histocompatibility testing workshop 1977

from F. Kissmeyer-Nielsen

The seventh (and largest) International Workshop in Histocompatibility was held in Oxford on 4-10 September, 1977. It was organised by Professor W. Bodmer (University of Oxford) with the assistance of Dr P. Morris (Oxford), Professor R. Batchelor (Queen Victoria Hospital, East Grinstead), Dr H. Festenstein (London Hospital Medical College) and Dr J. Bodmer (Oxford). The proceedings will be published by Munksgaard, Copenhagen in *Histocompatibility Testing 1977*, edited by W. Bodmer *et al.*

KNOWLEDGE of the major histocompatibility system of man, the HLA system, has advanced rapidly in recent years. It includes a still increasing number of closely linked loci, each with multiple alleles, controlling cell surface determinants important for humoral and cellular immunity. The biological importance of this genetic region is, furthermore, substantiated by the close association between some of the genes belonging to this region and an increasing number of diseases. It is known that the genetic information for HLA is on chromosome 6 and effectively includes the genes for the complement factors C2, C3, C4 and Bf as well as the erythrocyte systems Rodgers (Rg) and Chido (Ch). The polymorphic enzymes phosphoglucomutase-3 (PGM₃) and glyoxalase (GLO) are also on chromosome 6 but clearly separated from HLA. Knowledge of the genetic map of chromosome 6 is rapidly increasing, primarily because of the tremendous polymorphism of the HLA system which makes nearly every family informative in segregation analyses.

The rapidly increasing knowledge of the HLA system has depended heavily on the stimulus provided by seven international workshops in histocompatibility between 1964 and 1977.

The main subjects of the seventh workshop were:

(1) Serological investigations of the human Ia (=immune associated) determinants present on B lymphocytes and not on T lymphocytes.

(2) The genetics of these Ia determinants as revealed by family studies, including recombinants, and the relationship between the Ia specificities as detected by serological techniques

and the Dw determinants as revealed by typing in mixed lymphocyte cultures (MLC) using homozygous typing cells (HTC).

(3) Serological investigations of 'old' and new specificities belonging to the HLA-A, B and C loci.

(4) Investigations of a limited number of diseases in order to show whether they were more closely associated with Ia determinants than with HLA-(A and) B antigens and D determinants.

One hundred and fifty laboratories from all over the world participated allowing antisera for the definition of Ia determinants and HLA-A, B and C antigens to be tested against all major racial groups. For family studies data for more than 3,500 family members was included in the workshop; they were all tested with Ia antisera and most with HLA-A,B,C and some with HTCs to reveal Dw determinants. This resulted in a collection of nearly 800 haplotypes and the material included 122 recombinants of whom 42 provided information on Ia specificities.

The Ia specificities were generally found to be closely associated with the HLA-Dw determinants as identified with HTCs, but the Ia specificities as serologically identified on isolated B lymphocytes were generally somewhat 'broader' than the associated HLA-Dw determinants. Furthermore, in extensive family material no confirmed recombination between the Ia and Dw specificities was found. It is therefore still unclear whether the Ia specificities are identical to, or only closely associated with the Dw determinants. Biochemical investigations of the isolated gene products are in progress in various laboratories to resolve this question.

The 42 recombinants studied supported the map sequence of the HLA-loci on chromosome 6 as A, C, B and D.

As usual a WHO nomenclature committee met immediately after the workshop and discussed which specificities could be upgraded to full HLA-status from their previous provisional designation, indicated by a w before the number. HLA-A25 and A26 (splits of HLA-A10) and HLA-B15, B17, B37 and B40 were so upgraded.

Thirteen HLA-B specificities achieved provisional w-status and were designated Bw45 to Bw54. These specificities included 'splits' of previously designated specificities such as B5, B12, Bw21 and Bw22. The old broad specificities, 4a and 4b were named Bw4 and Bw6 as

they were found particularly useful in defining splits of 'broad' HLA-B antigens.

One new C-locus specificity, Cw6 (previously T7), was agreed on, and a total of six Cw-specificities can now be recognised, some of which are well enough defined to qualify for full HLA status. However, it was agreed, for the time being, to continue to designate them Cw1 to Cw6 in order not to risk confusion with the complement (-C-) factors.

The MLC typing allowed identification of five new Dw determinants Dw7 to Dw11, but all the Dw determinants remained provisional (w) as the correlations between some of the typing cells used for identification of the individual determinants were rather low, most probably because of inclusion phenomena and cross reactivity as known from the HLA-A, B and C serology.

The Ia specificities as established by serological assays on B lymphocytes were designated DRw followed by consecutive numbers from 1 to 7. It was clearly shown that these DRw specificities were highly associated with the Dw-related specificities, but it remains for future investigations to show whether they are identical to the Dw determinants.

HLA and disease

More than 2,500 patients were included in the workshop covering the following diseases: ankylosing spondylitis, multiple sclerosis, juvenile diabetes, psoriasis, active chronic hepatitis, idiopathic haemochromatosis, myasthenia gravis and rheumatoid arthritis and several others, to some of which DRw typing was applied for the first time.

Several of the diseases had previously shown associations with B locus antigens. In some cases the DRw association was roughly the same as the previously noted B locus association, for example, the relative risk for DRw3 in juvenile onset diabetes, myasthenia gravis and active chronic hepatitis was between 2 and 3 which was the level of risk for the antigen B8 with which DRw3 is in strong linkage disequilibrium. In the case of multiple sclerosis, the relative risk for DRw2 of 3.8 was similar to that found for the Dw2 antigen, and is much higher than the risk for B7 previously shown for this disease. The high association of A3 with haemochromatosis was confirmed with a relative risk of 9, this being of particular interest as it is the only disease so far showing a clear cut A locus association. A C locus association was found for psoriasis: Cw6, a newly defined antigen showed a relative risk of 4.3 in psoriasis and explained the previously noted associa-

F. Kissmeyer-Nielsen is Professor in Clinical Immunology, University of Århus, and Chief of the Blood Bank and Tissue Typing Laboratory, Århus Kommunehospital, Århus, Denmark.

tions with B13 and B17, with which Cw6 is in linkage disequilibrium.

Of greatest interest for the Ia serology were the associations found with DRw4 in juvenile onset diabetes and rheumatoid arthritis. In juvenile onset diabetes DRw4 has a similar relative risk to the previously noted B8 and DRw3 and obviously points to a complicated mode of inheritance for susceptibility. The association of DRw4 with rheumatoid arthritis is exciting because it is found in 60–70% of patients, as opposed to about 20% in the normal population. This approaches the frequencies seen for B27 for ankylosing spondylitis patients in early studies. With strict definition of ankylosing spondylitis now, the frequency of B27 approaches 100% and it may be that further studies on rheumatoid arthritis will reveal higher frequencies of DRw4 in some groups of patients. As a general conclusion it was clear that where patients had been Dw typed by MLC, very good agreement was found with the serological DRw typing and that the latter would be a preferable method for the studies of disease association with the HLA region, being simpler and less laborious.

Other activities

Special interest was focused on the possible relationship between DRw incompatibilities and results of human transplantations. Some groups reported on B cell cross matches before transplants and concluded that a positive B cell cross match seemed to be irrelevant to transplant survival, but the picture was obscured by the influence of 'cold' B cell active autoantibodies. Post-transplant investigations seemed to support a correlation between development of B cell active antibodies and rejection.

J. Dausset (Paris) and collaborators had DRw-typed 96 haploidentical skin grafts performed on family material 8 years ago. They confirmed their previously reported correlation with HLA-A and B incompatibilities and graft survival, but they also found a highly significant correlation between skin graft survival and the number of DRw incompatibilities. It is clear that further investigations in this area are needed.

Cell mediated lympholysis (CML) testing was the subject of an unofficial working group. The test involves education of cytotoxic lymphocytes (CTLs) in MLC-inducer cultures and subsequent testing against target cells in a chromium-51 release assay.

It has until now been dogma that the serologically defined HLA-A, B, C antigens are the actual targets for destruction in CML, while the influence of HLA-D determinants is unknown. There are, however, now

multiple indications that CTLs either recognise HLA-A, B, C antigens in a way different from that of antibody, or alternatively recognise determinants governed by loci other than HLA-A, B, C. There is even evidence of CML determinants controlled by chromosomal regions other than HLA, for example the male Y-chromosome, although killing through these determinants seems to be restricted by certain HLA antigens.

Thirty CTLs giving results inexplicable by HLA-A, B, C, D antigens have been located and correlated in an international CML workshop. Three tentative CML-defined specificities could be identified, and they may be of allelic genetic origin. Each of the putative CML-traits showed, however, correlations with HLA-antigens.

In conclusion, there may be reasons to believe that at least some CTLs produced *in vitro* recognise separate antigenic determinants, although much more work is needed before this statement can be finally substantiated.

The next workshop will be held in the US in about three years time and will be chaired by P. Terasaki. It will deal mainly with DRw and A, B, C serology, genetics of DRw, disease associations and transplantation. □

Somatomedins and related growth factors

from M. M. Rechler and S. P. Nissley

A workshop on somatomedins and other growth factors was held at the National Institutes of Health, Bethesda, Maryland, 19–21 September, 1977. The conference was sponsored by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Cancer Institute, National Institute of Child Health and Human Development and the John E. Fogarty International Center. The workshop was organised by M. M. Rechler and S. P. Nissley, NIH.

THE somatomedins are a group of polypeptides which are thought to mediate the effects of growth hormone on skeletal growth (see *News and Views* 267, 308; 1977).

M. M. Rechler is in the Diabetes Branch, NIMADD and S. P. Nissley is in the Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda.

At the workshop major advances were reported in the chemical characterisation of the various somatomedins. R. Humbel and E. Rinderknecht (University of Zurich) presented the complete amino acid sequence of insulin-like growth factor I (IGF-I), formerly known as NSILA I, one of the somatomedins purified from human plasma. IGF-I is a single chain peptide of molecular weight 7650. Its sequence is homologous with approximately 50% of the A and B chains of insulin; 17 of the 19 invariant residues of insulin are present in IGF-I. The region of IGF-I corresponding to the connecting peptide of proinsulin is shorter than that of human proinsulin (12 residues rather than 35). IGF-I also contains an 8-residue extension beyond the carboxy-terminus of the insulin A chain. Although the location of only one disulphide bond has been identified, the other four half-cystine residues align perfectly with the half-cystine residues in proinsulin. IGF-I shows approximately the same degree of sequence homology with both evolutionarily recent and primitive insulin molecules, leading Humbel and Rinderknecht to propose that IGF-I arose by gene duplication before the evolution of the vertebrates.

The relationship of IGF-I to the other somatomedins isolated from pooled human plasma remains to be determined. Rinderknecht and Humbel have previously reported that more than 70% of the 30 amino terminal amino acid residues are identical in IGF-I and IGF-II. Both IGF-I and II, and somatomedin C have isoelectric points of 8.2–8.6. Unlike IGF-I, somatomedin A is a neutral peptide and possesses only one half-cystine residue (L. Fryklund, AB Kabi, Stockholm). Fryklund also presented the complete amino acid sequence of somatomedin B. Although plasma levels of somatomedin B are growth hormone dependent, somatomedin B differs in chemical composition and biological properties from the other somatomedins. Somatomedin B has no sequence homology with insulin, consistent with its lack of insulin-like activity, but does show homology with protease inhibitors, and preliminary results indicate that somatomedin B has anti-trypsin activity.

A major enigma has been the relationship of the low molecular weight NSILA to NSILP (human serum non-suppressible insulin-like protein) purified by P. Poffenbarger (University of Texas, Galveston). NSILP is a glycoprotein of molecular weight 88,000 composed of two non-identical chains. The Zurich group finds that most of the non-suppressible insulin-like activity in plasma is found in a low molecular weight form following acid treatment

of serum whereas in Poffenbarger's purification scheme which avoids acid exposure, the large molecular weight NSILP accounts for all of the activity. R. Froesch (Zurich) presented the provocative observation that low molecular weight NSILA, combined with its serum binding protein (see below) and chromatographed on Dowex, is eluted as a higher molecular weight form that can no longer be dissociated by acid. Since Dowex chromatography is the first step in Poffenbarger's purification scheme for NSILP, Froesch proposed that NSILP may represent NSILA plus its binding protein.

Somatomedins have also been purified to homogeneity from a rat liver cell line (MSA, multiplication stimulating activity) and from rat serum (rat 'somatomedin'). MSA is a neutral peptide of molecular weight 8700 containing 3-4 half-cystines, carboxy-terminal glycine and a blocked α -terminus (A. Moses, NIH). Residues 19-38 of rat somatomedin, a basic peptide with three disulphide bridges distantly resemble the A chain of insulin and relaxin (W. Daudet, J. Jacobs, Washington University, St Louis). Rat somatomedin and MSA are clearly different polypeptides since they show no cross reactivity in specific radioimmunoassays (J. Daniels, Washington University, St Louis; P. Nissley, NIH).

Assays

The cross reactivity of the different peptides is so extensive that no assay yet exists that exclusively measures only one of the somatomedin peptides. Thus when these assays are used to determine 'somatomedin' levels in serum the composite effect of several peptides is being studied; the relative contribution of each somatomedin depends on the assay used.

Results from bioassays and competitive binding assays clearly establish that IGF-I, IGF-II, somatomedin A, MSA and probably somatomedin C constitute a class of closely related but not identical polypeptides. IGF-I, IGF-II, somatomedin A and MSA possess 1/50th-1/500th the activity of insulin in several *in vitro* assays in fat cells (J. Zapf, Zurich). These four purified somatomedins are also potent stimulators of DNA synthesis in chick embryo fibroblasts (Zapf; M. Rechler, National Institutes of Health). Somatomedin A and a preparation containing a mixture of IGF-I and IGF-II are equipotent in stimulating both uridine and sulphate incorporation into chick embryo cartilage; MSA was active but 10-20 times less potent (K. Gibson, Roche Institute of Molecular Biology, Nutley).

Collaborative studies (K. Hall, Karo-

linska Hospital, Stockholm; Rechler; Zapf) revealed extensive cross reactivity between IGF-I, IGF-II, somatomedin A and MSA in competitive binding assays in which 125 I-labelled homogeneous somatomedins bound to either the somatomedin receptors of various different cell types, or to a human serum carrier protein for somatomedin.

Autoantibodies against the insulin receptor isolated from a patient with a rare form of insulin resistance, block the binding of 125 I-MSA to somatomedin receptor on human skin fibroblasts suggesting that the somatomedin receptors on human fibroblasts share antigenic determinants with insulin receptors (Rechler).

Recently developed radioimmunoassays for the human somatomedins discriminate better among the somatomedin-related peptides than do bioassays and radioreceptor assays. R. Furlanetto and J. Van Wyk (University of North Carolina, Chapel Hill) reported that somatomedin A is approximately 3% as potent as somatomedin C in a somatomedin C radioimmunoassay. 125 I-MSA does not bind to the somatomedin C antibody. In a radioimmunoassay utilising IGF-I tracer and an antibody directed against IGF-I, somatomedin A is equipotent with IGF-I whereas IGF-II is 30-50 fold and MSA 500-fold less potent than IGF-I (Zapf). Hall presented preliminary data for a radioimmunoassay utilising 125 I-labelled somatomedin A tracer and antibody directed against somatomedin A in which IGF-I is approximately 10-fold more potent and IGF-II 10-fold less potent than somatomedin A. MSA does not cross react.

Binding proteins

Somatomedins are distinctive among polypeptide hormones in that they are associated with specific binding proteins in the circulation. There was general agreement that when radio-labelled IGF-I, IGF-II, somatomedin A or somatomedin C are incubated with human serum and analysed by gel chromatography, all ligands show a similar binding profile (Zapf; Fryklund; Van Wyk; D. Schalch, University of Colorado, Denver; R. Hintz, Stanford University). Most of the specifically bound tracer elutes near the position of albumin with a minority of the tracer being bound to a larger molecular weight protein (125,000-200,000). There was also general agreement that a 60,000 binding protein could be generated by acid treatment of the larger binding protein suggesting that the larger binding protein is an oligomer of the smaller one. In rats, both Moses and Zapf presented data showing that 125 I-MSA and 125 I-NSILA bind to large

molecular weight proteins in normal rat serum, and that both the binding profile on Sephadex G-200 and the binding capacity are growth hormone dependent. C. Meuli (University of Zurich), studying glucose uptake in rat heart perfused through the coronary arteries, found that addition of carrier protein to the perfusate abolishes the stimulation by NSILA. Meuli attributed this inhibition to an inability of the carrier protein-NSILA complex to diffuse readily out of the capillary bed. It is not known whether the *in vitro* anabolic actions of somatomedin are also inhibited by the binding protein.

Other growth factors

In a discussion of some of the non-somatomedin polypeptide growth factors R. Andre (Washington University, St Louis) presented data supporting a model of nerve growth factor (NGF) action in which NGF initially binds to cell surface receptors, is internalised by pinocytosis and ultimately binds to nuclear receptors. K. Lembach (Vanderbilt University, Nashville) described studies showing inhibition of 125 I-EGF (epidermal growth factor) binding to human skin fibroblasts by lectins specific for mannose, galactose and N-acetyl galactosamine residues, suggesting that the EGF receptor is a glycolipid or glycoprotein containing those residues. The carbohydrate nature of the EGF receptor was supported by data of R. Pratt and I. Pastan (NIH) showing that a Balb/c 3T3 mutant (AD6) which exhibits impaired biosynthesis of complex carbohydrates and glycoproteins due to a block in acetylation of Glc N-6-P, also shows decreased binding of EGF to cell surface receptors. AD6 cells grown in the presence of GlcNAc increased their binding of 125 I-EGF by 50%. D. Gospodarowicz (University of California, San Francisco) showed the complete amino acid sequence of brain fibroblast growth factor (FGF) and described the pathway by which a 169 residue inactive precursor molecule is converted into biologically active FGF consisting of residues 44-153. Gospodarowicz also reported that the entire FGF sequence is contained within the sequence of brain myelin basic protein. H. Antoniades (Harvard University, Boston) described the purification from serum of a heat stable cationic (pI 9.7) polypeptide of molecular weight 13,000. By radioimmunoassay this factor is present in small quantities in serum derived from platelet-poor plasma. A. Vogel and R. Ross (University of Washington, Seattle) reported the partial purification of a similar factor from platelets which they propose is the principal mitogen in blood serum responsible for the stimulation of DNA synthesis in tissue culture. □

review articles

The multi-disciplinary role of 'pion factories'

J. D. Davies*, C. J. Batty† & K. Green†

The multi-disciplinary role of intermediate energy proton accelerators in pure and applied nuclear physics is discussed with particular reference to the experimental programmes at LAMPF (Los Alamos Meson Physics Facility) and SIN (Swiss Institute for Nuclear Research, Zurich).

INTERMEDIATE energies, in the context of this discussion, refer to proton accelerator energies above the threshold for pi-meson production but below that for K-mesons, say 400 MeV up to 1 GeV. That is above the energies of the Van de Graff machines used for nuclear structure studies but below the multi-GeV proton synchrotrons used in high energy elementary particle physics. About 1960, experiments in this energy range were reaching the limits of technique with the then available intensities of the variable frequency synchrocyclotrons and so interest shifted to the new, higher energy proton synchrotrons where high energy particle physics has thrived. However, a very rich field remained, awaiting a new generation of proton accelerators with intensity increases of 3 to 4 orders of magnitude; the so called 'pion factories'. These machines have now been operational for a few years and it is an interesting time to review their role as multi-disciplinary facilities. Relevant parameters of these new accelerators are listed in Table 1 together with those of some older but recently upgraded machines. Duty cycle is an important but often neglected parameter in that it governs the type of experiment for which the machine is best suited.

The proton beams from these accelerators can be used in their own right but in general other particles such as pions, muons, protons, neutrons, electrons, photons and neutrinos are generated through proton interactions in massive targets. Some particle properties are listed in Table 2. Typical particle fluxes to be obtained from 100 μ A of proton beam are

$$\left. \begin{array}{l} 1 \times 10^{16} \text{ spallation neutrons at } \sim 1 \text{ MeV} \\ 1 \times 10^9 \pi^+/\text{s} \\ 1 \times 10^8 \pi^-/\text{s} \end{array} \right\} \text{ in the energy range } 100\text{--}600 \text{ MeV}$$

$$1 \times 10^7 \text{ stopping pions per s}$$

$$6 \times 10^6 \text{ stopping muons per s}$$

$$3 \times 10^{14} \text{ neutrinos per s at source}$$

The exact fluxes depend on the geometry of the beam concerned and in particular muon beams, which arise from pion decay, are very dependent on the exact layout. The highest intensity muon beam is at SIN¹ where a superconducting solenoid system collects and transports $3 \times 10^7 \mu^- \text{s}^{-1}$ at 125 MeV/c.

Such beams of particles are now being used for a wide range of experimental investigations, from the pure science aspects of nuclear and particle physics through their use as diagnostic tools in the applied fields of μ SR and mesic X-rays in condensed matter research to the directly practical fields of isotope production and

medical therapy. These fields will now be discussed in more detail with particular reference to characteristic experiments which are being carried out at the SIN and LAMPF meson factories.

Particle and nuclear physics

Particle and nuclear physics continue to play a central part in the programme of experiments although continually challenged by the increasingly important part played by the applied nature of much of the research. The weak interaction is being extensively studied at SIN and LAMPF and provides an ideal example of how the study of rare processes using the high fluxes from pion factories can successfully complement experiments at the high-energy machines which explore the sub-structure of 'elementary' particles. The conventional form for the weak interaction, with only vector and axial vector charged currents (V-A), has been shattered in experiments at high energies involving neutrinos and has opened up once again the whole question of the form of the weak interaction and the associated conservation laws. Muon decay experiments are free from strong interaction effects but are insufficient alone to specify the charged current weak interaction Hamiltonian. One needs to assume $m_{\nu_e} = m_{\nu_\mu} = 0$ and to look for indirect evidence from hadronic decays.

Limits on the neutrino mass are obtained by measuring momenta in the decay $\pi \rightarrow \mu + \nu_\mu$; presently a SIN experiment has set an upper limit² of 0.5 MeV for the mass but both SIN and LAMPF expect eventually to reach 0.2 MeV. Possible scalar and tensor contributions can be studied through the electron polarisation from muon decay. Limits will be reduced by a factor of 5 at SIN in experiments now under way.

Zero mass for the neutrino forces them to have 100% polarisation and the observed negative helicity accords with the charged current V-A Hamiltonian. The zero spin of the pion then forces its decay muon, or electron, to have the same, but unnatural, helicity and the decay can only proceed since the charged lepton mass is non-zero. The (V-A) form and electron-muon universality are sufficient to predict³

$$R = \frac{\pi^+ \rightarrow e^+ \nu_e}{\pi^+ \rightarrow \mu^+ \nu_\mu} = 1.233 \times 10^{-4}$$

The accuracy of the present experimental value³ of $R = (1.274 \pm 0.024) \times 10^{-4}$ will soon be improved by experiments at LAMPF and TRIUMF. Departure of experiment from theoretical prediction would also occur for example if a heavy lepton (L), whose existence has recently been indicated in high-energy experiments was coupled only with the electron neutrino. Furthermore if the heavy lepton is mixed with the muon and electron then the decay $\mu^\pm \rightarrow e^\pm \gamma$ could occur at a rate 10^{-9} to 10^{-10} of the normal decay. Such a decay, forbidden by separate, additive lepton conservation laws would be of fundamental significance and experiments are in progress at all the meson

*Physics Department, University of Birmingham, Birmingham, UK

†Rutherford Laboratory, Chilton, Didcot, UK

Table 1 Meson facilities

Facility	Accelerator type	Country	Range of proton energy (MeV)	Beam intensity (μA)		Duty cycle
				Planned	Operated at	
LAMPF	Linear	USA	600–800	1000	100	6×10^{-2} with 0.5 ns per 5 ns
SIN	Cyclotron	Switzerland	590	100	100	1.0 with 1 ns per 20 ns
TRIUMF	Cyclotron	Canada	180–520	100	50	1.0 with 1 ns per 42 ns
SREL	Synchro-cyclotron	USA	600	1–2	1–2	0.5
DUBNA	Synchro-cyclotron	USSR	680	25–50	2	0.5
CERN	Synchro-cyclotron	Switzerland	600	10	2	0.5

factories to search for this decay. The published limit⁴

$$B = \frac{(\mu^+ \rightarrow e^+ \gamma)}{(\mu^+ \rightarrow e^+ \nu \bar{\nu})} < 2.2 \times 10^{-8}$$

has already been reached at SIN and TRIUMF and should be considerably improved in the coming months by experiments at all three laboratories.

The high intensities of the pion factories are now sufficient to give good counting rates in high-resolution spectrometers in nuclear scattering experiments. Until now complex nucleus scattering experiments were restricted to well separated final states such as the ground state of ^{12}C with its first excited level at 4.4 MeV. The resolution is now sufficiently good to determine the energy state of many recoil nuclei. LAMPF are at present commissioning high-resolution spectrometers for both pions and protons.

At SIN, elastic and inelastic $\pi^\pm$ scattering from ^{12}C and the double charge exchange reaction $^{18}\text{O}(\pi^+, \pi^-)^{18}\text{Ne}$ have been measured⁵; in the latter they were able to resolve the ground state and 1.89 MeV first excited states, a reaction first reported⁶ from LAMPF in an experiment where a resolution of 4 MeV was insufficient to separate the states. Double charge exchange can now become a powerful tool in nuclear structure research, identifying $\Delta T_z = 2$ isobaric analogue states and giving exciting prospects for looking at proton rich nuclei.

Also at SIN is a high resolution pair spectrometer with large acceptance. This measures the energies of γ -rays coming from radiative pion capture $N(A, Z)(\pi, \gamma)N(A, Z-1)$, which comprises $\sim 2\%$ of all captures, and enables studies to be made of the energy levels of the final unstable nucleus⁷.

At LAMPF there has been considerable work⁸ on low-energy elastic $\pi^\pm$ scattering from selected nuclei, which conclusively demonstrated the importance of both nuclear structure and pion dynamics, and their nucleon–nucleon programme is such that it is only a matter of time until a complete set of experiments will have been done on elastic scattering, measurements of the spin-dependent parameters, and all inelastic channels.

Exotic atoms

A negative muon or pion will be slowed down in matter in a time of the order of 10^{-10}s , which is considerably smaller than the particle's lifetime. Being negatively charged it can be captured into an atomic orbit about the positively charged nucleus. The atomic levels for a given set of quantum numbers are increased in energy by the ratio of the particle mass to electron mass compared with the corresponding electron orbits whilst the classical radii of the atomic orbits are decreased by the same factor. This 'exotic' atom will become de-excited to orbits of lower principal quantum number (n), first by Auger transitions and later by X-ray emission. A muon eventually reaches the $n = 1$ orbit where it either decays or is captured by the nucleus. Because of the strong interaction a pion will be captured by the nucleus as soon as it reaches a state of low enough angular momentum where the pion wavefunction has a significant overlap with the nucleus. Measurement of these X-ray spectra can yield a wealth of information ranging through studies of quantum electro-dynamics (QED) and nuclear structure to studies of atomic cascades and applications to chemical analysis.

Precise measurements of X-ray energies for muonic atoms made 3 to 4 years ago seemed to indicate a discrepancy⁹ with values

calculated using QED. Improved measurements made more recently¹⁰ together with further refined evaluation¹¹ of the 'vacuum polarisation' terms in the QED calculations now give very satisfactory agreement. Measurements of X-ray energies for muonic atoms are still of great interest, however, as the values for the lower n states can give valuable information on the nuclear charge distribution. Experimenters at CERN, LAMPF and SIN are collaborating in very precise measurements of muon transitions in $A \sim 40$ to $A \sim 95$ isotopes¹². This is providing a systematic study of nuclear charge distributions and its changes with Z and N . Measuring hyperfine splittings gives information on nuclear electric multipole moments and work of this type¹³ is being done at LAMPF for the osmium and platinum isotopes.

In the field of applications the use of muonic X-rays for *in-situ* analysis of bulk material composition is being investigated^{14,15} at LAMPF. The method is non-destructive and by varying the beam parameters it is possible to investigate the distribution of elements through the volume of the object. The method has the advantage that it can be used for any element and is of particular interest for light elements where methods such as neutron activation are not suitable. In the case of medium and high Z elements it is sometimes possible to distinguish between isotopes of the same element. Typical sensitivities are of the order of a few parts per thousand.

So far we have assumed that the relative concentrations of different elements can be deduced from measurements of the relative X-ray intensities. However, it is now known that the chemical and physical form can influence the relative intensities of X rays from exotic atoms of a particular element. This is because the particle is initially captured into an atomic state of large radius which can be affected by the atomic and molecular electron clouds and hence by the chemical and physical form of the material. Thus studies of mesic X-ray spectra could yield considerable information about the structure of the outer electron shells and there are extensive programmes of work to study these phenomena at several laboratories. At present the subject is an interesting branch of atomic physics but the improved and systematic experiments now in progress will require detailed comparisons with realistic theoretical models before exotic atom chemistry can become a serious and reliable tool for chemical and solid state studies.

What additional information is obtained by studies of pionic X-rays? As already mentioned the strong interaction with the nucleus causes the pions to be captured if their wavefunction has a significant overlap with the nucleus. As a result the lowest observable level will be broadened and shifted in energy from the QED values and measurements of these effects can be a powerful tool¹⁷ for investigations of pion-nucleus interactions. This is a topic of particular significance because the pion-two-nucleon interaction is expected to dominate. Capture on single nucleons

Table 2 Particle properties

Particle type	Mass (MeV)	Spin ($\hbar$)	Lifetime (s)	Principal decay mode
Neutron	939.6	$\frac{1}{2}$	918	$p + e^- + \bar{\nu}$
Proton	938.3	$\frac{1}{2}$	Stable	—
Pion	139.6	0	2.6×10^{-8}	$\mu + \nu$
Muon	105.7	$\frac{1}{2}$	2.2×10^{-6}	$e + \nu + \bar{\nu}$
Electron	0.511	$\frac{1}{2}$	Stable	—
Neutrino	~ 0	$\frac{1}{2}$	Stable	—

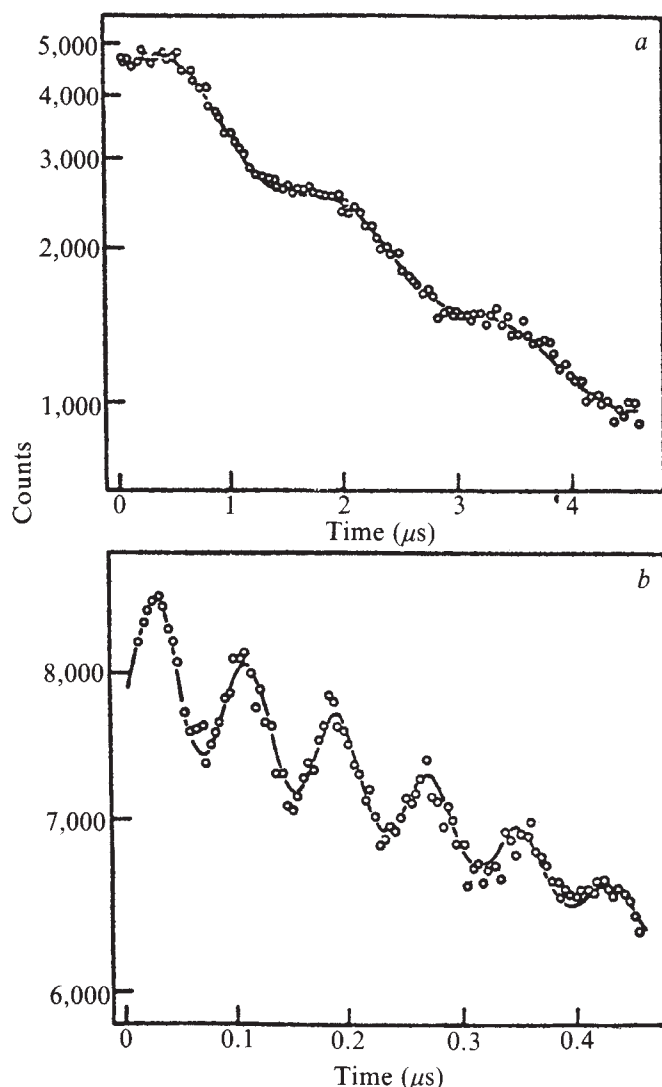


Fig. 1 Muon precession signal in paramagnetic (a) and ferromagnetic (b) Ni. This figure is based on one in the article "On the application of polarised muons in solid state physics" by A. Schenek³².

bound in a nucleus is strongly suppressed by energy and momentum conservation.

A very wide range of measurements of shifts and widths of pionic X-ray transitions are now available. The high intensity stopped pion beams make measurements with separated isotope targets possible so enabling comparisons to be made between different isotopes of the same element.

Biomedical applications

There has been considerable development work at many accelerators on the use of negative pions for the treatment of cancer. Clinical studies with human patients started in 1974 at LAMPF¹⁸ and SIN¹⁹ are building a large medical centre with treatment of patients planned for 1979. Negative pions have physical and biological properties that could lead to significant improvements in radiation therapy which presently is used for more than half of cancer patients.

A negative pion can be made to pass through normal tissue with low ionisation dose and then to stop in a small well defined volume with high dose. This high dose results from both the increase in ionisation rate as the pion slows down and the short range fragments coming from the stopped pions which annihilate on a nucleus; a significant fraction of the pions rest mass of 140 MeV goes into producing heavy nuclear ions. Anoxic (oxygen deficient) cells commonly found in tumours have an intrinsic resistance to damage by radiation but which can be overcome by high linear

energy transfer (LET). The high ionisation rates of stopping pions and nuclear fragments may help to increase their relative biological efficiency (RBE).

Several laboratories have dedicated biomedical pion beams with intensive and detailed programmes designed to lead to the treatment of patients. Starting with simple systems, radiobiologists are investigating the claims for high LET, the effects on normal tissue and genetic damage. At LAMPF, treatment of selected human patients¹⁸ began in 1974 and has now included tumours in skin, subcutaneous tissue, muscle, oral cavity, chest wall and lung. Measurements on the pion beams and the stray radiations, physical dose in different tissues and equivalent dose measurements with biological dosimeters are some of the physical and biological dosimetry studies being made.

The well known EMI X-ray scanner provides density reconstruction in axial tomography of tissues for medical diagnosis. At LAMPF an experiment has started using the energy loss of a proton passing through a specimen to determine the density projections; computer simulation has suggested that 0.4% density resolution can be obtained with a proton dose several times smaller than from X rays.

Isotope production

When a complex nucleus receives energy from a 600 MeV proton or from a pion or muon, it can fragment, and many particles are given off—alpha particles, neutrons, protons and spallation nuclei. Several systematic studies have started, particularly at LAMPF: often the initial reason is pure nuclear physics but frequently applications soon follow.

Of considerable interest are the nuclear and atomic properties of proton- and neutron-rich nuclei far from the mass stability curve that are produced in (p, p Yn) and (p, Xp n) reactions. In studies of very short lived nuclei, the on-line mass isotope separator of the ISOLDE facility²⁰ at CERN has a major role. The subject also goes hand-in-hand with isotope production for medical and industrial use.

Reactors make neutron-rich products cheaply whilst compact cyclotrons make neutron-deficient isotopes, via (p, Yn) reactions, (where Y = 1, 2, 3) which are relatively free of contaminants. The two sources and types of isotope essentially do not compete. For nuclear medicine application neutron-deficient isotopes will almost always be preferable since they generally decay by electron capture. Where positrons are emitted, a very attractive form of radiography becomes available with the almost back-to-back annihilation γ rays providing a characteristic signature.

For higher energy protons both production yields and Y increase whilst (p, Xp Yn) reactions also become significant. However, this is a nonspecific reaction with many neutron-deficient isotopes being made but specific decay routes can often overcome this disadvantage. Many useful isotopes can only be made from 800 MeV protons and many attractive isotopes are more easily and cheaply made. For instance²¹, at LAMPF, protons on molybdenum yield many useful isotopes of zirconium, yttrium and strontium. At SIN, they are investigating²² the medically important ¹²³I.

Radiation damage and neutron production

The copious production of low-energy neutrons and spallation products in 800 MeV proton interactions allows the use of these machines for fundamental studies on radiation damage at a high rate. Such damage is a major engineering problem in fission and fusion reactors, especially in the first walls of a possible fusion reactor. Heavy ions are the usual investigatory tool but only for the surface. Through the spallation process a proton beam can be effectively a source of heavy ions throughout the body of the material studied.

So far there have been extensive calculations²³ and one experiment^{24,25} on aluminium samples irradiated to a damage level of a few displacements per atom (d.p.a.) which were compared with fission neutron damage of Al to the same d.p.a. The following exciting conclusions were reached. 800 MeV proton bombardment simulates fission and fusion neutron irradiation but

with a much lower heat per d.p.a. so that high current densities or damage rates are possible. Similar void densities for a given d.p.a. were obtained with proton and fission neutron irradiation although the former produces 1,000 times as much He. This is a very significant result since there had been speculation that the high He production per d.p.a. of fusion neutrons compared with those from fission could give a corresponding increase in the void nucleation rate. Additionally, there was no unexpected proton damage although a wide range of impurity atoms was produced.

Neutrons are numerically the greatest product in 800 MeV proton interactions. Each proton can produce 24 neutrons from a tungsten target or 40 from one of uranium; in some sense there are more neutrons from spallation than from fission. In Canada there is extensive interest in using spallation neutrons from intermediate energy accelerators for fertile to fissile conversion. Radiative neutron capture in ^{238}U and ^{232}Th leading through β decay to ^{239}Pu and ^{233}U are being studied at TRIUMF in the FERFICON²⁶ project.

Muon spin rotation

Muon spin rotation (μSR), so named because of its close analogy with nuclear magnetic resonance (NMR) and electron spin resonance (ESR), performs many functions of these well-tried techniques in the study of condensed matter but in addition has many advantages of its own²⁷. The method originated in experiments on the $(g-2)$ and magnetic moment of the muon. The former has tested quantum electrodynamics to better than 1 part in 10^8 and demonstrates that muons behave as heavy electrons. At SIN they are determining the muon magnetic moment to 1 part per million by measuring its Larmor precessional frequency in a known magnetic field.

The principle of μSR is to determine the Larmor precession frequency and the relaxation time of polarised muons by observing the muon decay asymmetry as a function of time. This field pioneered in the 1970s by the Berkeley group at the 700 MeV cyclotron is an ideal example of the cross-fertilisation of the ideas between nuclear particle physicists and condensed matter research. There are two kinds of muon spin rotation, μ^+ SR and μ^- SR; in the former the muon behaves as a light proton and in the latter it behaves as a heavy electron. In the former case the μ^+ diffuses interstitially through the material whilst for the latter the μ^- will be bound in the ground state of a muonic atom and hence located close to the nucleus.

A typical experiment of this type involves slowing down and implanting in the sample one of these muons whose spin direction is well defined. The process of thermalising the muons occurs on a short enough time scale ($\sim 10^{-10}\text{s}$) such that the full spin polarisation of the muon is preserved. The muon spin will then precess about the local magnetic field at a frequency determined by this field and the muon magnetic moment until such time as it decays $\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_\mu$. The probability for the muon to decay at a given time is a constant and leads to a characteristic exponential decay curve for the muon with a time constant of $2.2\mu\text{s}$. The positron (e^+) is emitted in the decay preferentially along the muon spin direction so that observation of this positron implies the direction of the muon spin after a time (t). Observations of many such muons, all decaying at different times (t) leads to a curve of the type shown in Fig. 1. As can be seen the characteristic exponential decay of the muon has superimposed upon it an oscillation whose frequency is dependent upon the local magnetic field seen by the muon. The difference between paramagnetic and ferromagnetic nickel is dramatic and obvious.

The advantages to be gained in using μSR as compared with other techniques like NMR, ion implantation, Mossbauer effects or even neutron diffraction in solid state physics are based on the fact that the muon is point-like with no structure or significant electromagnetic perturbations; its spin is $\frac{1}{2}\hbar$ and therefore it has no quadrupole moment. The fact that 10^6 to 10^7 muons are sufficient to give an adequate signal and with only a few muons present in the sample at any one time means that cross talk and disruption or radiation damage to the host is minimal.

Uses to which this technique can be put are many and varied but include studies of magnetic effects²⁸, diffusion processes and of impurity centres in semiconductors and insulators. For example at SIN positive muons are being used to look at diffusion effects in metals as a function of temperature²⁹ and to study atomic defects. In the latter case the muon has considerable advantages over positrons, which can also be used. Because of its greater mass the muon can be used to detect traps with a potential too weak to find a positron and in certain cases absolute defect concentrations can be measured. A similar programme of experiments has been proposed at LAMPF.

Muonium (μ^+e^-) is formed in the slowing down of positive muons in non-metallic materials, particularly in liquids. The existence of these thermal sources of relatively stable bound muons allows chemical effects to be studied. For example at SIN comparisons³⁰ are being made between muonium (mass $\sim 1/9 m_H$) reaction rates with those for the corresponding H atom rates. Previous studies of isotope effects were limited by the 1:3 mass ratio between ^1H and ^3H .

Neutron sources

We have tried to show in this review that a high-intensity intermediate energy proton accelerator can not only be used for the traditional studies in nuclear and elementary particle physics but can also support a full, wide-ranging and rewarding multi-disciplinary programme of experiments. The meson factories at LAMPF, SIN and at TRIUMF in Canada, all conceived with the pure research aspects of nuclear and particle physics in mind, are now assuming an increasingly important role in these applied aspects. One such topic which is becoming important at LAMPF and at TRIUMF and which is being considered around the world for future generations of meson factories is the use of intermediate energy accelerators as primary sources for the generation of very high neutron flux densities, greater than those available from conventional nuclear fission reactions.

The use of neutrons to study the structure of matter is now a well established technique and Europe, with Britain well to the fore, has a wide programme of work in this field using reactor facilities both nationally and at the French, German and British Institute Laue-Langevin in Grenoble, France. The condensed matter research community in their striving for ever more intense and versatile sources have now realised that a proton accelerator could be the basis of the next generation of facilities for this type of work. We have already mentioned that through the spallation process, a 800 MeV proton can give up to 40 neutrons when it strikes a uranium target. This, together with the fact that the proton beam can be pulsed, so enabling time-of-flight methods to be used, could give a neutron source with between one and two orders of magnitude improvement over that currently available from the best reactors.

Such a proton based source has been proposed at the Argonne laboratory in the USA, in the USSR, whilst in Britain the Science Research Council has just received approval for a similar project to be built at the Rutherford Laboratory. This latter project³¹ will use much of the present NIMROD accelerator now engaged in high-energy physics research and which is due to be closed down in mid 1978. The new facility in its basic form will provide 200 μA of 800 MeV protons in 53 pulses per s each of 200 ns duration. In principle such a machine could also be used for experiments other than neutron scattering. It is to be hoped that these new machines will embody flexible duty cycles as part of their design criteria and enable the full range of multi-disciplinary uses of an intermediate energy accelerator to be carried out at the one facility: the cross fertilisation of ideas being of benefit to the whole community. The programmes of research at SIN, LAMPF and TRIUMF have shown the way for the community to benefit from the expensive and sometimes remote world of high energy particle and nuclear physics.

Reports on proposed experiments and work in progress are given in Annual Reports from the SIN and TRIUMF laboratories and in Newsletters from LAMPF and SIN. We have used these sources extensively in the preparation of this article.

- ¹ SIN Newsletter 6, 4 (1976).
- ² Daum, M. *et al.* *Phys. Lett.* **60B**, 380–384 (1976); *SIN Newsletter* 8, 26 (1977).
- ³ Bryman, D. & Picciotto, C. *Phys. Rev.* **D11**, 1337 (1975).
- ⁴ Parker, S., Anderson, H. L. & Rey, C. *Phys. Rev.* **133B**, 768–778 (1964).
- ⁵ SIN Newsletter 8, 22–23 (1977).
- ⁶ Marks, T. *et al.* *Phys. Rev. Lett.* **38**, 149–152 (1977).
- ⁷ Alder, J. C. *et al.* *Proceedings Conference on Meson-Nuclear Physics*, A.I.P. Conference Proceedings No. 33, 624–625 (1976).
- ⁸ Amann, J. F. *et al.* *Phys. Rev. Lett.* **35**, 426–429 (1975).
- ⁹ Dixit, M. S. *et al.* *Phys. Rev. Lett.* **27**, 878–881 (1971).
- ¹⁰ Tauscher, L. *et al.* *Phys. Rev. Lett.* **35**, 410–412 (1975).
- ¹¹ Watson, P. J. S. & Sundarsen, M. K. *Can. J. Phys.* **52**, 2037–2059 (1974).
- ¹² Shera, E. B. *et al.* *Phys. Rev.* **C14**, 731–747 (1976).
- ¹³ Los Alamos Report LA-6553-PR, 74–75 (1976).
- ¹⁴ LAMPF Users Newsletter 9, No. 1, 59–61 (1977).
- ¹⁵ Hutson, R. L. *Los Alamos Report LA-5867-MS* (1975).
- ¹⁶ Gershtein, S. S. & Ponomarev, L. I. *Part III Muon Physics* (eds Hughes, V. W. & Wu, C. S.) 142–233 (Academic, New York and London, 1975).
- ¹⁷ Backenstoss, G. A. *Rev. nucl. Sci.* **20**, 467–508 (1970).
- ¹⁸ Kligerman, M. M. *Proceedings of the International Conference on Cyclotrons and their Applications* 419–426 (Birkhauser, Basel, 1975).
- ¹⁹ *Proceedings SIN Pion therapy workshop* (unpublished).
- ²⁰ *CERN Courier* **16**, 256 (1976).
- ²¹ Grant, P. M., Kahn, M. & O'Brien, H. A. *Jour. Inorg. Nucl. Chem.* **37**, 413–417 (1975).
- ²² Hegedus, F. & Peek, N. F. *SIN Physics Report No. 171* (1976).
- ²³ Coulter, C. A., Parkin, D. M. & Green, W. V. *J. nucl. Materials* **67**, 140–184 (1977).
- ²⁴ *Lamp users newsletter* 9, 61–63 (1977).
- ²⁵ Los Alamos Report LA-6678-RR, 96–97 (1977).
- ²⁶ TRIUMF Annual Report 45 (1975).
- ²⁷ Brewer, J. H., Crowe, K. M., Gyax, F. N. & Schenk, A. *Part III Muon Physics* (eds Hughes, V. W. & Wu, C. S.) 3–139 (Academic, New York & London, 1975).
- ²⁸ Graf, H. *et al.* *Phys. Rev. Lett.* **47**, 11–14 (1977).
- ²⁹ Camani, M. *et al.* *SIN Newsletter* No. 7, 23–23 (1976).
- ³⁰ Percival, P. W. *et al.* *Chem. Phys. Lett.* **47**, 11–14 (1977).
- ³¹ *Rutherford Laboratory Report RL-77-064/C* (1977).
- ³² Schenk, A. in *Nuclear and Particle Physics at Intermediate Energies* (ed. Warren, J. B.) (Plenum, New York, 1976).

Basic and applied research at the TRIUMF meson factory

M. K. Craddock, K. L. Erdman* & J. T. Sample†

The TRIUMF 520 MeV H⁻ cyclotron produces intense beams of protons, pions and muons supporting basic research in nuclear, particle and solid-state physics, nuclear chemistry and biomedicine, and applied research in electromagnetic breeding of nuclear fuel, proton radiography, radioisotope production and cancer treatment.

'MESON factory' is the term coined to describe intermediate-energy accelerators designed as prolific sources of pions and muons. So far three capable of delivering proton currents larger than 100 μ A have come into operation^{1,2}: LAMPF, an 800 MeV proton linear accelerator at Los Alamos, New Mexico (1972); SIN, a 590 MeV proton cyclotron, near Zürich (1973); and TRIUMF, a 520 MeV H⁻ cyclotron, in Vancouver (1974). These facilities have proved to be of multidisciplinary interest. Nuclear physicists and chemists can study the interactions of the nucleus either with pions, the particles mainly responsible for holding protons and neutrons together, or with the nucleons themselves, at energies where their de Broglie wavelength is much smaller than a nuclear diameter. Particle physicists can study the properties of nucleons, pions and muons and their interactions among themselves. Solid state physicists can use muons as novel probes for determining magnetic fields in crystal lattices, and neutrons for scattering and diffraction studies of molecular structure. Chemists can use muonium (μ^+ e⁻) atoms in the study of chemical reactions in gases. Biomedical scientists can study the effects of the various radiations on living cells. The meson factories also have worthwhile practical applications, particularly in medicine. The localised dose delivered by negative pions holds out the hope for improved treatment of deep-seated cancers. Proton-induced spallation reactions can be used to produce useful proton-rich radioisotopes and to convert fertile to fissile nuclear fuel. Protons can also be used for high resolution, low dose, radiography.

TRIUMF is a cooperative project of the University of Alberta, the University of British Columbia, Simon Fraser University and the University of Victoria—the acronym having been too precious to drop when the original three universities were joined by a fourth. The land, buildings and some administrative services have been provided by the universities; the accelerator and experimental equipment were funded by the Atomic Energy Control Board of Canada from 1968 to March 1976, when responsibility was transferred to the National Research Council of Canada.

The cyclotron and particle beams

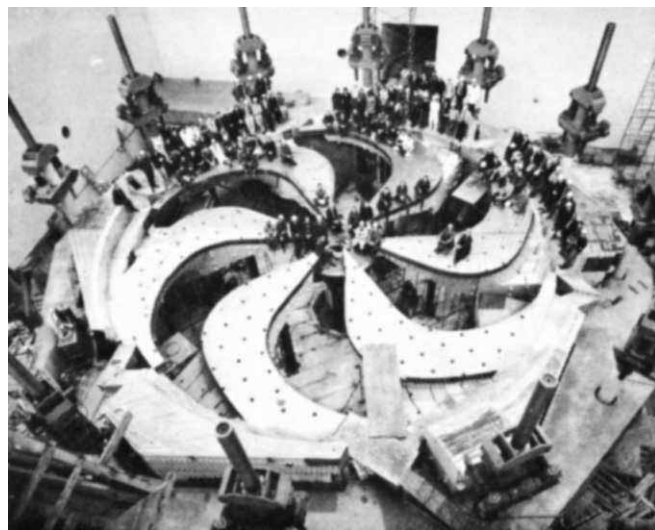
The design of TRIUMF³ is based on a proposal⁴ by J. R. Richardson (director 1971–76) for a meson factory based on a sector-focusing cyclotron accelerating H⁻ ions. The chief advantages of this design are (1) continuous, rather than pulsed, beams

are produced with modulation only at the RF frequency; (2) the extracted beam energy is continuously variable from 180 to 520 MeV; (3) two or more beams can be extracted simultaneously at independent energies and intensities.

The first feature is not only advantageous for the coincidence detection of nuclear reaction products, but is also the essential characteristic making possible the acceleration of 100 μ A beams, rather than the 1 μ A typical of the (pulsed) synchrocyclotrons previously available in this energy range. Continuous operation at a fixed frequency is made possible by the division of the magnet into sectors (Fig. 1). These provide periodic focusing forces on the internal beam, thus compensating the vertical defocusing associated with a magnetic field in which the ion orbit time is the same at all energies.

The unique feature of the TRIUMF design, which is crucial to obtaining multiple beams and variable energy, is the acceleration of H⁻ ions. Extraction, a notoriously difficult and inefficient

Fig. 1 The TRIUMF staff assembled on the six lower sectors of the cyclotron magnet, Spring 1972. The 12 columns carry jacks for lifting the entire upper half of the magnet, weighing 2,000 tons.



*Physics Department, University of British Columbia and TRIUMF, Vancouver, B.C., Canada

†TRIUMF, Vancouver, B.C., Canada

Table 1 Beam properties

Beam line	Particles	Polarisation	Energy (MeV)	Momentum spread	Intensity	Spot size (cm × cm)
1/4	p	0	180–520	0.2%	100 μA	0.3 × 1.4
1/4	p	70–80%	180–520	0.2%	120 nA	0.3 × 1.4
4A	n	40–75%	(160)–500	(1%)	$6 \times 10^7 \text{ s}^{-1}$	7 × 9
M8	π^+		20–120	1.5–15%	$\leq 10 \times 10^8 \text{ s}^{-1}$	$\begin{Bmatrix} 1 \times 2 \\ 10 \times 10 \end{Bmatrix}$
M9	π^+		15–65	2–15%	$\leq 3 \times 10^8 \text{ s}^{-1}$	3 × 10
M9	π^+		stopping	2–15%	$5 \times 10^7 (\text{s-g-cm}^{-2})^{-1}$	
M9	μ^+	60%	20–75	2–15%	$\leq 10^7 \text{ s}^{-1}$	3 × 10
M9	μ^+	100%	4.1	2–15%	$2 \times 10^6 \text{ s}^{-1}$	5 × 5
M20	μ^+	100%	4.1	5%	$5 \times 10^5 \text{ s}^{-1}$	6 × 6
M20	μ^+	60%	90	5%	10^6 s^{-1}	10 × 10
M11	π^+		(30–350)	(0.1%)	$(\leq 8 \times 10^8 \text{ s}^{-1})$	(1 × 1)

Intensities of secondary beams are quoted for the proton currents listed. π^- and μ^- beams have the same properties as π^+ and μ^+ beams, except that their intensities are a factor ~ 5 lower. Figures in parentheses represent theoretical estimates.

process for circular accelerators, is made trivial by passing the negative ions through a thin carbon or aluminium foil⁵, stripping the two electrons away and leaving positively-charged protons, which then curve away from the cyclotron. In the case of TRIUMF two foils are used, 180° apart in azimuth, to provide two external beams, and each may be adjusted in position to provide beams of continuously variable energy and intensity. By changing foil shapes as well, it has been possible to vary the ratio of intensities in the two extraction channels from 1:1 to 1:5000.

A full report of recent developments on the cyclotron and beam lines (Fig. 2) has been given by Dutto *et al.*⁶. In regular operation beam currents have been limited to 10 μA up to now by the capacity of the temporary beam dump—although over 100 μA was run for 45 minutes in a successful test in July 1977. 100 μA beams should be regularly available by the end of the year when the permanent beam dump and thermal neutron facility (TNF) should be ready for operation. These will be capable of handling the 135 kW power in the 300 μA beams which could be extracted at 450 MeV. The TNF, with a lead target in a D₂O moderator, will be a potent source of thermal neutrons ($\sim 5 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$) for scientists of various persuasions in the reactor desert of Western Canada.

Three secondary beams of pions and muons are currently produced at target T2 in the high intensity beamline BL1. The performance of these is summarised in Table 1. Three further lines are under construction: BL1B for low intensity protons, M11 for fast pions, and M13 for slow pions and muons (Fig. 2).

In addition to the regular ion source, a ‘Lamb shift’ polarised source has been operating since February 1976. It can provide 960

nA of H⁻ ions, of which 120 nA can be accelerated and extracted with 75–80% reversible polarisation. The variable energy of this polarised beam makes it unique in the 200–500 MeV region; such is the demand for experiments that it has been scheduled for almost half the time since it became operational. For the past year a beam of almost monoenergetic polarised neutrons has also been available⁷. When the polarised proton beam hits a liquid deuterium target the neutrons emerging at 9° in the initial polarisation-beam plane are found to be between 40% and 75% polarised, depending on the initial proton energy (Fig. 3).

Developments planned for 1978 include extraction of proton beams in the 70–180 MeV range for isotope production and lower energy nuclear experiments, and the addition of a third harmonic component to ‘flat-top’ the RF wave. This will improve the beam quality and allow individual turns to be extracted with a consequent reduction in energy spread from the present 1 MeV FWHM to only 0.1 MeV, sufficient to distinguish many nuclear states.

Basic research with pions and muons

An experiment of considerable topical interest this year has been a search on the M9 channel for muon decays by the process $\mu^+ \rightarrow e^+ + \gamma$. In 1964 the branching ratio for such decays, which lack the neutrinos appearing in the usual process $\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_\mu$, was found to be $< 2.2 \times 10^{-8}$. This has been taken as evidence for the conservation of separate electronic and muonic lepton numbers. Recently, however, leptons heavier than the muon have been found⁸. If new leptons were to be coupled to both electrons and muons the conservation laws need not be exact

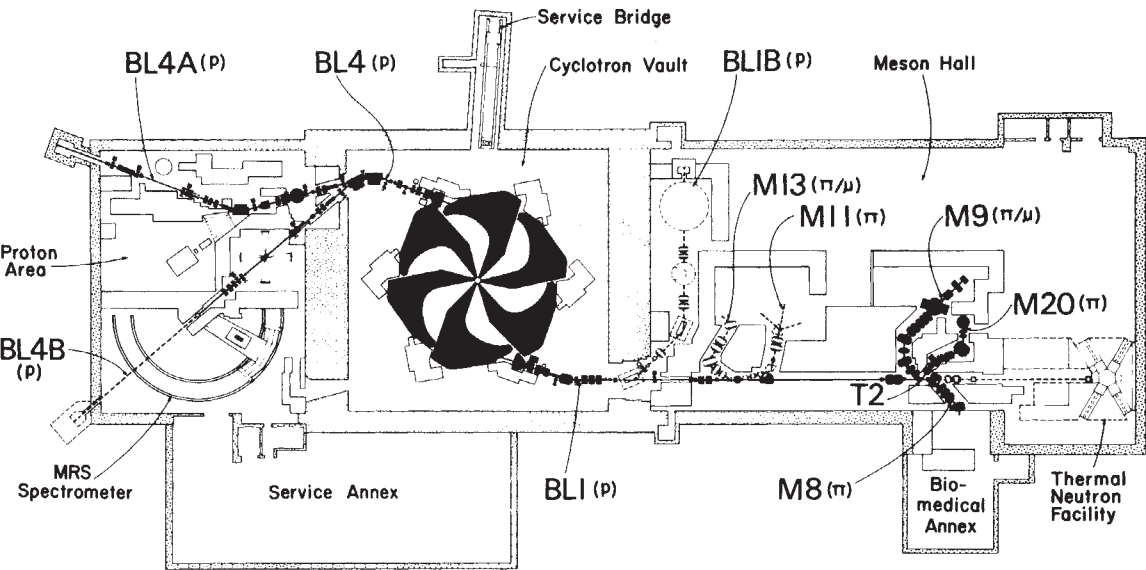


Fig. 2 Layout of the facility. Existing beam lines are indicated by solid lines, beam lines planned for future installation by dashed lines (see text).

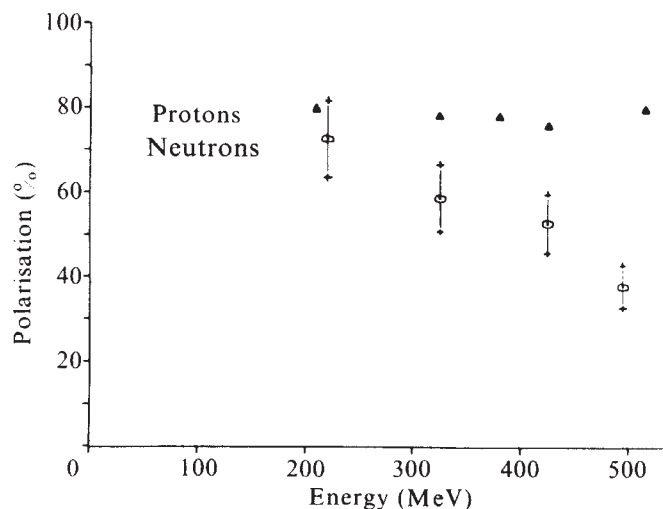


Fig. 3 Polarisation of the polarised proton (▲) and neutron (○) beams at various energies. The neutron values represent the spin rotation parameter R_1 for the $d(p,n)2p$ reaction multiplied by an assumed polarisation of 0.78.

and a finite fraction of muons could decay by the $\mu \rightarrow e\gamma$ process (see, for example, ref. 9). An alternative mechanism¹⁰ which could permit $\mu \rightarrow e\gamma$ decays is exchange of Higgs bosons, particles postulated in modern gauge theories of the weak interaction but not yet observed experimentally. Theoretical estimates for these processes have put the branching ratio for $\mu \rightarrow e\gamma$ in the range $10^{-12} \rightarrow 10^{-8}$.

To identify the decay process experimentally, two large sodium iodide crystals (46 cm and 36 cm diameters) were placed on opposite sides of the stopping target to measure the energies of electrons and γ rays. Analysis of the first runs has revealed one possible event and one background event, yielding an upper limit of 3.6×10^{-9} on the branching ratio at a 90% confidence level¹¹.

Measurements are also being made of the branching ratios for the rare decays of the pion $\pi \rightarrow e\nu$ and $\pi \rightarrow e\gamma$. The former ratio provides a sensitive test of muon-electron universality, that is whether the muon and electron respond equally to the weak interaction.

Information on the threshold pion-nucleon (π -N) interaction has been obtained from studies of stopped pion charge exchange (π^- , π^0) reactions in hydrogen, deuterium and ^3He . At very low energies the 'strong' pion-nucleon (π -N) force is in fact relatively weak; its comparability to electromagnetic interaction strengths is demonstrated by the near-equality of the rates for the stopped pion charge exchange reaction $\pi^-p \rightarrow \pi^0n$ in hydrogen and the radiative capture reaction $\pi^-p \rightarrow \gamma n$. The 'Panofsky ratio' of these two rates has now been remeasured at TRIUMF using the large sodium iodide crystals to identify the π^0 s by their two decay γ rays. The value obtained¹² was 1.546 ± 0.009 ; the precision of this result, twice that of previous work, helps to determine the π -N interaction at threshold more accurately.

In deuterium the charge exchange reaction $\pi^-d \rightarrow \pi^0nn$ at rest is strongly suppressed by conservation laws. In fact the non-observation of this reaction provided early evidence that the π^0 had the same (negative) parity as the π^- . The reaction is not totally prohibited, but for it to occur both the π^0 and the two neutrons must be produced with non-zero orbital angular momentum. With only 1.09 MeV kinetic energy available such a final-state configuration is very unlikely. Using information from the π -N interaction at threshold the branching ratio has been estimated¹³ to be $1.49 \pm 0.10 \times 10^{-4}$. The sensitivity of the present equipment has now enabled the reaction to be seen at rest for the first time¹³ (Fig. 4) with a branching ratio of $1.45 \pm 0.19 \times 10^{-4}$, an order of magnitude smaller than the upper limit previously established, and in agreement with the theoretical estimate.

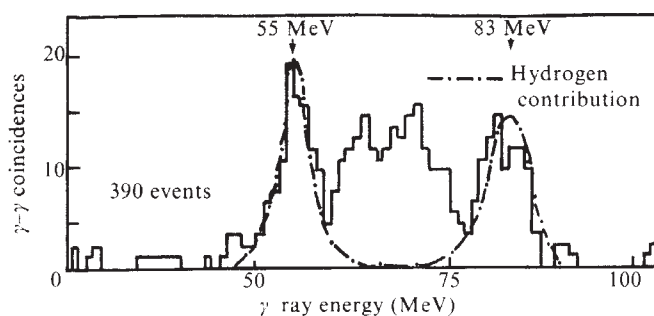
Studies of π -nuclear interactions are exemplified by an experiment on the elastic scattering of positive pions from ^{12}C . This has

utilised the good quality low energy pion beams available on the M8 channel to study the relatively unexplored region below the (3,3) resonance, caused by excitation of individual nucleons to the $\Delta(1232 \text{ MeV})$ state, and which dominates the scattering of pions between 70 and 400 MeV. Measurements were made¹⁴ with 30, 40 and 50 MeV pions at scattering angles from 15° to 150° . This angular range includes the sensitive region of interference between Coulomb and nuclear scattering at these energies and should therefore provide a stringent testing ground for any theoretical models. In fact theoretical calculations¹⁴ based on free π -N scattering are in poor agreement with the data unless nuclear absorption and Pauli exclusion effects are included; these effects have not been needed to account for data at higher energies. When 30 MeV negative pions were scattered from the same target the data clearly showed the difference between π^+ and π^- Coulomb interference effects.

Muons which slow down and stop in matter will generally be captured by nearby atoms in the few microseconds before they decay. The events which take place in this short period make possible two fields of study, muonic X-rays and muon spin rotation (μSR). Both are being actively pursued at TRIUMF, but but we shall concentrate on the latter. The muons in the beam are strongly spin-polarised as a result of conservation of angular momentum at their formation in pion decay ($\pi \rightarrow \mu\nu$), the neutrino being inherently polarised while the pion has spin zero. The standard μSR technique consists in measuring the time interval between the stopping of a spin-polarised muon and the detection in some fixed direction of the electron emitted in its decay $\mu \rightarrow e\nu\bar{\nu}$. Since the electron tends to come off in the direction of the muon spin axis, a time spectrum accumulated from many μ decay events will display oscillations with the frequency of precession of the muon spin in the local magnetic field.

μSR studies involve three groups on channel M20. One group has concentrated on the behaviour of positive muons in ferromagnetic metals, where the muons are captured into interstitial positions in the lattice but can then diffuse from site to site. In cobalt (Fig. 5) temperature variations in the relative directions of internal dipolar and hyperfine fields lead to drastic changes in the μ^+ precession frequency and relaxation rate¹⁵. In a large extremely pure single crystal of iron ($150 \times 48 \times 1.1 \text{ mm}$) precession was observed down to 23 K in zero applied field¹⁶; previously it had not been seen below 100 K. The results suggest high temperature diffusion of the μ^+ by thermally activated hops, superseded below 44 K by quantum tunnelling between sites. A second group uses 4.1 MeV 'surface' muons to produce muonium (μ^+e^-) atoms (Mu) in gaseous targets. When impurities such as Cl_2 , HBr and O_2 are added the muonium precession signal shows a concentration-dependent quenching rate from which chemical reaction rate constants can be extracted, thus allowing a comparison of the chemistry of Mu, H and D atoms. The third group has found that Mu atoms formed when muons stop in fine silica powder diffuse rapidly out of the grains into the vacuum (J. B. Warren, personal communication). This paves the way for new studies using silica powders as a 'moderator gas' for chemistry experiments. In the realm of particle physics this discovery will be

Fig. 4 180° coincidence γ -ray spectrum obtained using two large NaI detectors, showing the characteristic signatures of the stopped pion charge-exchange reactions $\pi^-p \rightarrow \pi^0n$ (wings) and $\pi^-d \rightarrow \pi^0nn$ (centre—here observed for the first time).



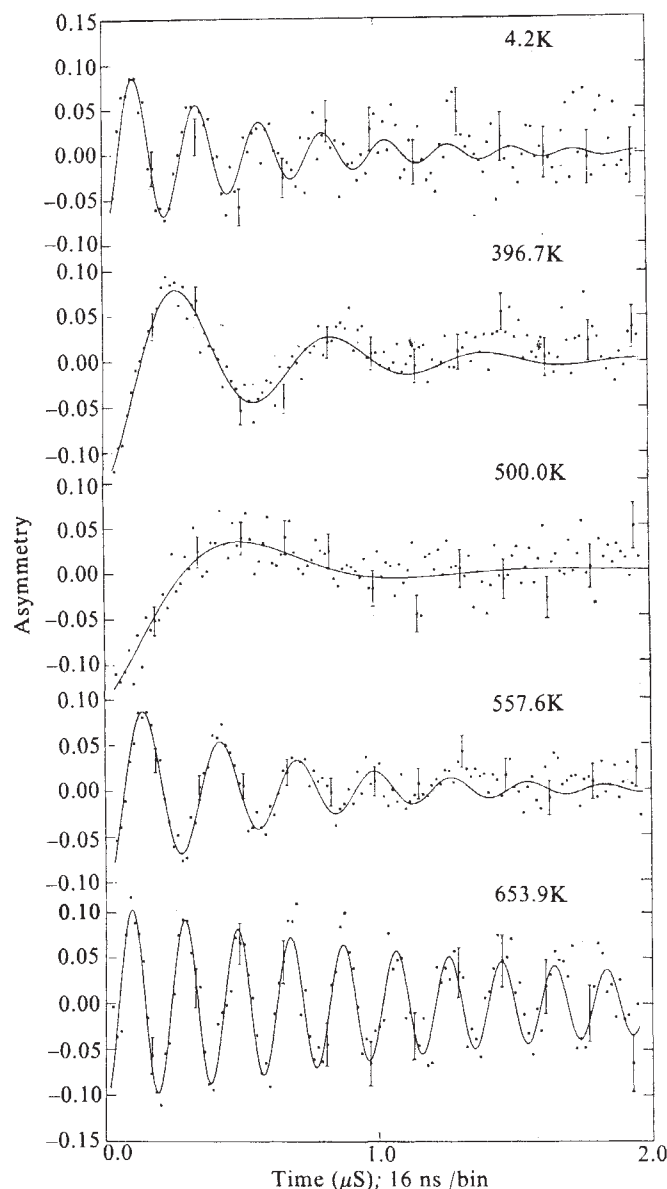


Fig. 5 Precession signals from positive muons in cobalt over a range of temperatures (with the muon decay folded out). The temperature variations in precession frequency and relaxation rate are directly related to variations in the relative directions of the internal dipolar and hyperfine magnetic fields in the cobalt.

applied to a search for muonium-antimuonium conversion in vacuum.

Basic research with protons and neutrons

The availability of intense, variable energy, beams of protons and neutrons makes the study of nucleon-induced reactions an important aspect of the basic research programme at TRIUMF. The most fundamental of these reactions is of course the elastic scattering of one nucleon (N) by another, and this is the subject of a comprehensive series of measurements on beamline 4A. Because the force between two nucleons depends on the relative directions of their spins it is necessary to use either a spin-polarised beam or target if the spin dependence of the force is to be completely determined. In fact nine independent parameters are needed to describe N-N scattering above the inelastic threshold at 280 MeV, rather than the one needed for spin-zero particles.

Initial measurements have concentrated on the Wolfenstein parameters describing the changes in polarisation on scattering. For pp scattering polarised protons were directed into a liquid hydrogen target and their subsequent polarisation was measured using a large high-efficiency polarimeter. With the aid of a superconducting spin-precession solenoid the parameters P , D , R and R' were determined at a number of forward angles for five

energies between 209 and 515 MeV. These data have greatly improved the accuracy to which isospin $T = 1$ N-N scattering is known at these energies¹⁷.

To study np scattering a polarised neutron beam was developed, as described above, to avoid the inaccuracies inherent in extracting pn from pd scattering data. In the process of this work the polarisation transfer parameters D_t , R_t and R'_t for $d(p,n)2p$ were measured⁷. (Compare Fig. 3). Measurements of the angular distribution of P and D_t in np scattering have been completed at a number of energies. The 325 MeV np data, together with the purely $T = 1$ pp data, has enabled a long standing ambiguity in the $T = 0$ N-N scattering to be removed¹⁸.

Pion production through the (p,π^+) reaction near threshold has been studied using both polarised and unpolarised proton beams on hydrogen, deuterium, beryllium and carbon targets. A 50 cm Browne-Buechner spectrograph was used to analyse pions with laboratory kinetic energies between 15 and 90 MeV. In the case of hydrogen ($pp \rightarrow d\pi^+$) the measurements¹⁹ for the first time give clear evidence of the importance of d-(as well as s- and p-) wave effects for pions with centre-of-mass energies from 60 MeV down to only 8 MeV.

For 200 MeV protons on ^9Be and ^{12}C surprisingly high peak asymmetries ($-65 \rightarrow -95\%$) were observed²⁰ for pions associated with both the ground state and groups of excited states of the residual nucleus; for instance, for the three states of ^{13}C at 3.09, 3.68 and 3.85 MeV the unresolved pion asymmetry was -95% at 60° . There is so far no theoretical explanation for these high negative values.

The scattering and reactions of protons on light nuclei are being studied on line 4B. At several hundred MeV the protons in the beam have much higher energies and speeds than those of the nucleons in a nucleus, which are therefore essentially stationary during an interaction. Furthermore the de Broglie wavelength of the incoming proton is smaller than the nuclear diameter so that it tends to interact with single or a few neighbouring nucleons rather than with the nucleus as a whole. The intermediate energy region is therefore a good one in which to test theoretical attempts to predict p-nucleus scattering from p-nucleon scattering, and to study pairing and clustering of nucleons. All in all the phenomena to be expected are rather different from those familiar at lower energies.

Experimentally, $p\text{-}^4\text{He}$ scattering has been measured at both forward ($4\text{--}14^\circ$) and backward ($140\text{--}168^\circ$) angles. A surprise has been the high (85%) analysing power at 160° at 400 MeV. Another interesting polarisation effect was the observation²¹ of J-dependence in $(p, 2p)$ cross-sections for 200 MeV polarised protons incident on ^{16}O (events involving proton knockout from the $p_{1/2}$ shell could be separated from those from the $p_{3/2}$ shell). In other experiments proton quasi-free and proton-deuteron quasi-elastic scattering from ^{12}C have been studied. To improve the analysis of reaction products a medium resolution (0.5 MeV) spectrometer is being commissioned on this beam line. This matches the present $\Delta E = 1$ MeV (FWHM) energy spread of the primary beam and is adequate to separate reactions proceeding via different excited states of many nuclei. A second stage is planned to improve this spectrometer resolution to 0.1 MeV to match the beam ΔE of 0.1 MeV expected from separated turn operation of the cyclotron.

Nuclear chemistry studies in beam line 4 concern proton-induced light fragment emission and proton-induced fission. For the identification of the fragments a 5-detector 'fragment telescope' has been developed which is effective up to oxygen. Comparing the energy distribution of fragments from Ag for 200 to 500 MeV bombarding energies with those reported at 1 to 5 GeV it seems that the evaporation mechanism satisfactory at higher energies will not explain the 200 MeV data (R. G. Korteling, personal communication). Studies of proton-induced fission have used a gas jet radioactivity transport system. A time-of-flight spectrometer has also been commissioned. Proton-induced fission of uranium and spallation of iodine have provided neutron-rich and neutron-deficient Sb isotopes, whose β - and γ -ray emissions are being studied.

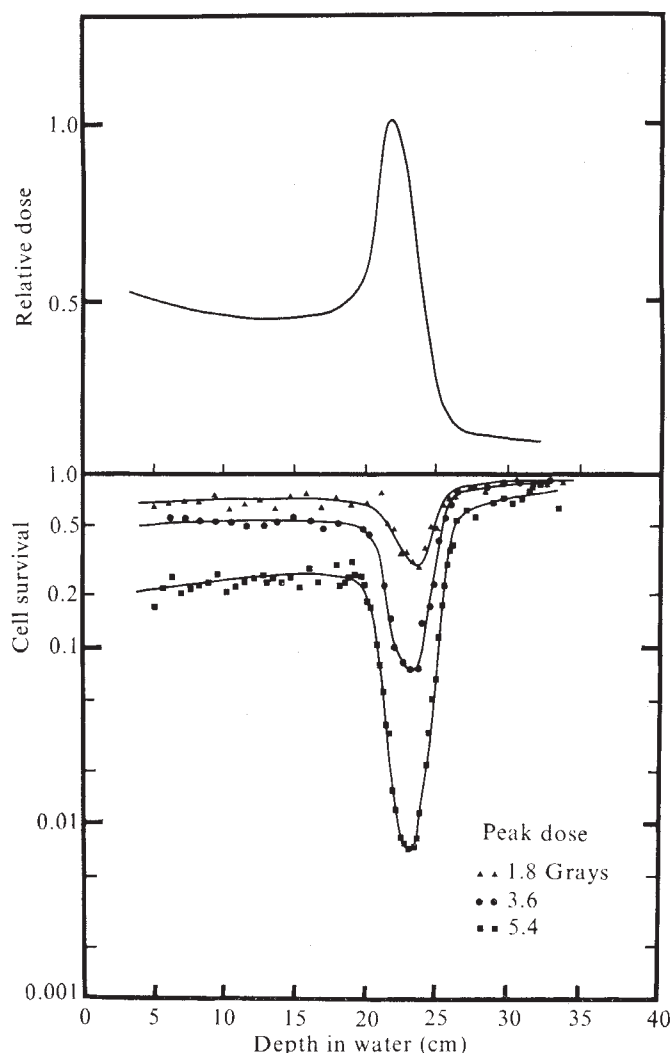


Fig. 6 Survival of Chinese hamster cells (CH₂B₂) π^- -irradiated in 25% gel/medium. Top: The depth dose profile of the 90 MeV pion beam. Bottom: Survival profiles for cells irradiated in gel/medium at 0°C. Dose rate at peak approximately 2 rad min⁻¹. Control plating efficiency 70%.

Biomedical use of negative pions

Although the *raison d'être* of TRIUMF is research in basic science, an active programme is under way to explore possible applications of the various beams. At the moment the biggest of these projects is an investigation of the potentially favourable properties of negative pions for treating cancer, funded jointly by the British Columbia Cancer Foundation, Health and Welfare Canada, and the National Cancer Institute of Canada. Compared with neutral X-rays and γ -rays whose interactions fall off exponentially with depth and which therefore give their greatest dose near the skin, negative pions have a definite range in matter and give their greatest dose where they stop—mainly because of the local deposition of their rest mass energy (140 MeV) in 'pion stars' on absorption in a nucleus following capture into atomic orbits. Pions are therefore expected to be more effective for treating deep-seated tumours. Furthermore, the high linear energy transfer (LET) component of the radiation field produced by the pion stars decreases the oxygen enhancement ratio (OER) and thus reduces the inherent radioresistance of hypoxic tumour cells²². Clinical investigation of pion radiotherapy has had to await the coming into operation of intense pion beams (say $10^8 \pi^- s^{-1}$ or more); for the M8 channel at TRIUMF this would require a primary beam of 100 μA protons. The lower currents which have been available so far have, nevertheless, been useful

for preparatory studies. They fall into three general areas: measurement of the clinically important properties of the pion beam, dosimetry of the pion beam and biological measurements *in vitro* of the relative effectiveness of pion radiation²³.

The stopping π^- flux in the M8 channel has been found to peak for 100 MeV pions; in water these have a range of 29 cm, within which 50% are lost by nuclear or electronic interactions. Sextupole magnets in the channel enable the spot size to be adjusted from $1.2 \times 1.8 \text{ cm}^2$ to $4 \times 15 \text{ cm}^2$ or $10 \times 10 \text{ cm}^2$ while maintaining a uniform distribution. Contamination of the beam by muons is unimportant; electrons are dominant at low energies, but at 100 MeV the e^-/π^- ratio is only 0.18.

Dose fields in a water phantom can be automatically scanned in three dimensions under computer control through a CAMAC interface; the computer is also used to monitor the beam position and magnet settings, and to collect and display the data. A depth-dose profile for a 90 MeV pion beam is shown in Fig. 6. For a wide momentum bite the width of the peak can be increased to about 8 cm H₂O. The best available beam tune provides about 20 rad h⁻¹ μA^{-1} protons over a volume of $3 \times 3 \times 5 \text{ cm}$ in the stopping region. For measuring the RBE and OER factors in cell survival experiments, a gel suspension technique has been developed²⁴ which avoids the mixing problems of fluid media.

Measurements *in vitro* of cell survival, using this technique, were begun in 1976 when the proton current was raised to 5 and then 10 μA . The results of irradiating Chinese hamster cells (CH₂B₂) with peak doses of 1.8, 3.6 and 5.4 Grays are shown in Fig. 6. From these preliminary measurements it seems that the biological properties of pion radiation differ little from those predicted. Measurements on biological systems *in vivo* have begun and clinical investigations will start approximately six months after the current is raised to 100 μA .

Applied research with protons

Three possible applications of proton beams are under study at present. Two of these—proton radiography and radioisotope production—are of medical interest; the third concerns the electromagnetic breeding of nuclear fuel.

The use of protons for radiography depends on their well defined range in matter. A detector placed near the end of the range of a monoenergetic proton beam will see large changes in intensity for only small changes in the thickness or density of the material traversed. For 200 MeV protons the enhancement factor can be as high as 50. A promising application for this 'marginal range radiography' is in the localisation of small tumours in soft human tissue, particularly as the dose would be relatively low. With a view to such applications preliminary experiments have been started at TRIUMF using a 200 MeV proton beam collimated down to 2 mm diameter (E. W. Blackmore, personal communication). The pencil beam is scanned in a raster fashion across the target using steering magnets under computer control. The protons transmitted may be detected on film or in a range telescope consisting of four thin plastic scintillators. Initial tests have been encouraging, showing, for example, that 0.25 mm steps in aluminium or perspex can readily be resolved. Work is now proceeding towards the development of a system capable of scanning over a $10 \times 10 \text{ cm}^2$ area in 10 s for a total dose of less than 100 mrem.

Work on radioisotope production has concentrated on the development of a viable technique for producing ¹²³I (J. S. Vincent, personal communication). This is a highly desirable isotope for diagnostic nuclear medicine because its decay by electron capture enables it to deliver a specific dose only 1% of that from the conventional, β -emitting ¹³¹I. 480 MeV protons are allowed to bombard a caesium heat pipe target producing xenon isotopes (among others) by spallation reactions; the caesium is heated above its melting point, freezing the xenon atoms which are then floated 18 m in a helium jet to a liquid nitrogen trap. After three ¹²³Xe half-lives the trap is warmed to remove long-lived Xe isotopes; a further delay clears ¹²¹I, when the remaining iodine is distilled off from the daughter isotopes. The only significant contaminant is ¹²⁵I, present at the 0.3% level at

the end of bombardment. Unfortunately, this gives a specific dose only a little less than ^{131}I , and it is much longer lived (59 d) than ^{123}I (13 h). It is computed to double the dose from ^{123}I (that is, from 1% to 2% of that from ^{131}I) in 28 h.

With the cooperation of Vancouver General Hospital 5 mCi of ^{123}I produced in this way was used for a whole-body search for a metastatic thyroid tumour in a patient; the resolution was found to be superior to that from an ^{131}I scan. It is now proposed to build a pilot plant for the regular production of 300 mCi ^{123}I per week.

The third proton application under study is the electromagnetic breeding of nuclear fuel. Under contract to Atomic Energy of Canada Ltd, an experimental programme has begun to study the conversion of 'fertile' ^{238}U and ^{232}Th to fissile ^{239}Pu and ^{233}U respectively under proton bombardment. The high energy protons serve to produce an intense flux of spallation and evaporation neutrons; the conversion then proceeds through radiative neutron capture and two β -decays. The first step in this study (I. M. Thorson, personal communication) has been to measure the neutron flux produced when 350 and 450 MeV protons are stopped in small (2.5 to 4.0 cm diameter) cylinders of uranium and thorium. For this purpose the target is surrounded by a 1.8 m diameter water tank in which the thermal neutron flux distribution is measured. The total neutron capture rate in the water can then be estimated and the appropriate correction applied to

the integrated flux. The next step will be to determine the fertile-to-fissile conversion rates by measuring characteristic γ -rays emitted in the decay steps. These measurements will begin this autumn.

We thank all our colleagues at TRIUMF and the participating universities whose work we have had the privilege of reporting.

1. Michaelis, E. G. *IEEE Trans. NS-22*, 1385-1396 (1975).
2. Hagerman, D. C. *IEEE Trans. NS-24*, 1605-1610 (1977).
3. Vogt, E. W. & Richardson, J. R. *IEEE Trans. NS-13*, (4) 262-276 (1966).
4. Richardson, J. R. *Nucl. Instr. Meth.* **24**, 493-500 (1963).
5. Rickey, M. E. & Smythe, R. *Nucl. Instr. Meth.* **18**, 66-68 (1962).
6. Dutton, G. *et al. IEEE Trans. NS-24*, 1653-1655 (1977).
7. Amsler, C. *et al. Nucl. Instr. Meth.* **144**, 401-406 (1977).
8. Perl, M. L. *et al. Phys. Rev. Lett.* **35**, 1489-1492 (1975).
9. Cheng, T. P. & Li, L.-F. *Phys. Rev. Lett.* **38**, 381-384 (1977).
10. Bjorken, J. D. & Weinberg, S. *Phys. Rev. Lett.* **38**, 622-625 (1977).
11. Depommier, P. *et al. Phys. Rev. Lett.* **39**, 1113-1116 (1977).
12. Spuller, J. *et al. Phys. Lett.* **67B**, 479-482 (1977).
13. MacDonald, R. *et al. Phys. Rev. Lett.* **38**, 746-749 (1977).
14. Johnson, R. R. *et al. Nucl. Phys.* (in the press).
15. Nishida, N., Nagamine, K., Hayano, R. S., Yamazaki, T., Fleming, D. G., Duncan, R. A. & Brewer, J. H. *Hyperfine Interactions* (in the press).
16. Nishida, N. *et al. Solid State Commun.* **22**, 235-239 (1977).
17. Axen, D. A. *et al. Lettere al Nuovo Cimento* **20**, 151-156 (1977).
18. Amsler, C. *et al. Phys. Lett.* **69B**, 419-421 (1977).
19. Jones, G. *Proc. 1st Int. Conf. Nucleon-Nucleon Interaction* (American Institute of Physics, New York, in the press).
20. Auld, E. G. *et al. Bull. Am. Phys. Soc.* **22**, 590-591 (1977).
21. Kitching, P. *et al. Phys. Rev. Lett.* **37**, 1600-1602 (1976).
22. Raju, M. R., Gnanapuran, M., Richman, C., Martins, B. I. & Barendson, G. W. *Br. J. Radiol.* **45**, 178-181 (1972).
23. Henkelman, R. M., Skarsgard, L. D., Lam, K. Y., Harrison, R. W. & Palcic, B. *Int. J. Rad. Oncol., Radiat. Phys.* **2**, 123-127 (1977).
24. Skarsgard, L. D. & Palcic, B. in *Proceedings of the 23th International Congress of Radiology*, **2**, 447-454 (Excerpta Medica, Amsterdam, 1974).

articles

Primaeval melting of the Moon

S. Keith Runcorn

Institute of Lunar and Planetary Science School of Physics, The University, Newcastle upon Tyne, UK

Leona Marshall Libby

Environmental Science and Engineering Department

Willard F. Libby

Department of Chemistry, and Institute of Geophysics and Planetary Physics, and Environmental Science and Engineering Department, University of California, Los Angeles, California 90024

There is evidence that the Moon melted completely 4,400 Myr ago, and between 4,000 Myr and 3,200 Myr ago had an internal magnetic field. But gravity could not have provided the heat of melting, and it must have come from short lived radioelements. Theory suggests the transuranics with atomic numbers between 114 and 126 may be relatively stable, and it is shown that these 'superheavy elements' fit the requirements of the early heat source in the moon.

THERE is widespread evidence for primaeval melting and differentiation processes in the Solar System, for example, iron and some stony meteorites have melted. The Earth has an iron core which, from evidence of its past magnetic field, is at least 2,000-Myr-old, and has had a sialic (Si-Al) crust since 3,600 Myr ago. While geochemistry suggests that the core formation and differentiation occurred very quickly, in 100 Myr, after the earth's accretion, the arguments are inconclusive. Melting produced extensive lava flows, generated at depth on Mars, Mercury and Venus. Some crustal deformation and core

differentiation must have occurred, as small present-day magnetic fields are present. The asteroid Vesta also has a melted surface. But, except for certain meteorites, the dates of these meltings and the completeness of the differentiation are uncertain so it could be argued that the differentiations of the terrestrial planets could have been due to heating long after accretion had been completed. Nevertheless, the ubiquity of melting in the solar system is of great significance.

With large bodies like the Earth and Venus, it is possible that the final stages of accretion release gravitational energy which, if trapped as heat, is enough to melt the body provided that the heat is retained, and not radiated to space, by opaque dust clouds around the accreting objects¹. Moreover, if melting did not take place during accretion, and the planet so formed was uniform in composition, the subsequent core formation would release comparable energy; in the case of Earth about twice as much as that released in its history from U, Th, and ^{40}K radioactivity². If the formation of the core were rapid, this energy would be adequate to heat Earth to its melting point and cause complete differentiation, although heat would be transported away by solid-state convection.

Other mechanisms of generating heat have been suggested; but as records of planetary melting and differentiation are fragmentary, evidence to decide between them does not exist. For example, Sonnett *et al.*³ suggested that in an early T-Tauri phase of the Sun, intense solar wind magnetic fields could have heated the planets by induction. As their ferromagnetic silicates are semi-conductors, the induced currents would cause their electrical conductivity to increase rapidly; thus considerable magnetic energy could be deposited in a planet. Such electromagnetic heating seems particularly applicable to melting meteorites, for induction by poloidal magnetic fields is limited by the skin effect to small depth. But heating of stony meteorites would have driven out xenon; and as xenon⁴ is still present there is a problem with this theory.

Therefore, although Earth has had, and other terrestrial planets may have had, for much of their histories molten cores and solid but slowly convecting mantles, their early thermal histories remain obscure. In contrast, we know a great deal about the first 1,000 Myr of the Moon's evolution⁵. The Apollo Moon landings have shown that a differentiation took place 4,400 Myr ago, producing the anorthositic highlands and the source region of the mare basalts⁶. They have shown that the moon's iron core was molten until at least 1,300 Myr had elapsed⁷, and that it must have an internal heat source sufficient to generate a lunar magnetic field. This dynamo field inferred from the palaeomagnetism of the crystalline rocks disappeared after 3,200 Myr, either because the core solidified or because the heat sources became inadequate to sustain dynamo action⁸.

We can, therefore, speculate on the primaeval sources of heat, which melted the moon in its first few 100 Myr to form its differentiated crust and iron core. We will show that for an object the size of the Moon the gravitational energy released in accretion and from known radioactive elements is insufficient. There have been predictions that relatively stable superheavy elements exist: if so, they might have been an important heat source in the early solar system. If evidence for their existence were to be found, the chemistry of the Moon, planets and cosmos will have to be re-examined.

The giant pleochroic haloes found in terrestrial micas⁹ have diameters of 150 to 200 μm ; if due to the emission of α particles this range implies energies up to 14 MeV, much more energetic than those from any known natural α -emitting nuclei. Gentry's¹⁰ explanation for this was that the rare earth inclusions within the haloes must have contained superheavy nuclei of which some fraction still exists. These monazite inclusions were bombarded by a proton beam and it was claimed¹¹ that among the X-ray lines excited, were those calculated for elements with $Z = 114, 116, 124, 125, 126$ and 127 . Other experiments which have followed have all been negative¹²⁻¹⁴, although only two of them, those at Stanford and Harwell¹⁵, have used monazite crystals which were taken from giant haloes. It is argued that these later experiments fall short of the required sensitivity due to inhomogeneous distribution of superheavy elements¹⁶. An alternative explanation of the pleochroic haloes has been proposed¹⁷: radioactive nuclei of $Z \sim 92$ emitting low energy α particles which, striking water molecules trapped in the mica or in the inclusions produce knock-on protons of great enough ranges to account for the discoloured spheres. To support this hypothesis von Wimmersperg and Sellschop¹⁷ assume that the inclusion contained a homogeneous mixture of monazite and water. We consider that mica could not contain enough water close enough to the inclusions to explain the spherical haloes. Furthermore, in his account of the phenomena of these giant haloes, Gentry¹⁰ shows photographs of micas in which giant haloes and normal haloes due to U decay exist side by side about 100 μm apart.

A second kind of evidence for the existence of superheavy nuclei is reported by G. N. Flerov¹⁸, who has studied spontaneous fission in meteorites and measured the number of neutrons emitted in each such fission. He found that the average number of neutrons per fission lies between 4 and 10, with 95%

confidence. This may be compared with < 3 for the known spontaneously fissioning elements. Thus it seems that the number of neutrons per spontaneous fission for nuclei in meteorites is much greater than for any known nucleus. The number of neutrons per spontaneous fission increases with mass, suggesting that superheavy nuclei are responsible.

A third kind of evidence suggests that the existence of superheavy elements explains the anomalous abundances of isotopes of xenon in carbonaceous chondrites which have a pattern inconsistent with yields from fission of U, Pu, Cm, and Cf^{19,20}.

Molten Moon and lunar magnetic field

We now develop our view that the moon is crucial for understanding early melting processes because its primaeval history has been preserved. Urey¹ presented convincing arguments that the Moon was formed by accretion, recognising that it was probably too small to have been melted by gravitational energy, and argued that it had remained undifferentiated and was therefore a key to the origin of the solar system. This insight is still fundamentally valid, even though fragments of anorthosite found in the Apollo sampling of the regolith, and identified as originating in the highlands showed that the moon had differentiated early to form a highland shell of anorthositic gabbroic composition²¹. Geochemists²² then calculated that only the top few hundred kilometres of the primitive Moon had to be melted to provide the observed thickness of this shell, and unlike the earth this differentiation could be securely dated; it occurred 150 Myr after the origin of the Moon. So, in the absence of conclusive evidence of the cause of melting, it seems reasonable to suggest, despite the uncertainties, that the cause was the last gravitational energy released when the Moon began to assume its present size⁶.

It has been widely assumed that the velocity of the incoming material would equal the escape velocity. For the Moon today, this is 2.3 km s^{-1} , so that the $2.8 \times 10^3 \text{ J g}^{-1}$ of material accreted would have been the energy released in the final stages of the moon's formation. As the energy required for melting silicates from 0 K is only $2 \times 10^3 \text{ J g}^{-1}$, it has been accepted that this energy, if none were lost, could have been effective in melting the outer part of the Moon.

There are also strong arguments suggesting that the Moon melted to the centre a few hundred Myr after its origin. The mare basalts have crystallisation ages between 3,200 and 3,900 Myr, but their Pb-Pb model ages are about 4,400 Myr (ref. 22). Rb-Sr systematics²³ and samarium-neodymium decay²⁴ give similar model ages which are interpreted in terms of a closed-system melting²⁵. The original differentiation, providing the source material of the lunar basalts, occurred about 4.4×10^3 Myr ago and later this melted again to provide the magma which filled the maria between 3.9×10^3 and 3.2×10^3 Myr ago. The closed-system melting leads to a geophysical model which has three shells: an outer anorthosite shell over a basalt-rich shell, below which is the olivine or pyroxinite mantle^{26,27} surrounding the core. The variations of electrical conductivity with depth, the seismic data²⁸, and the moment of inertia²⁹

Table 1 Isotope heating and melting of the Moon

Assumptions:
1. $C_p = 0.20 \text{ cal g}^{-1} \text{ } ^\circ\text{C}$
2. $\Delta H_{\text{fusion}} = 100 \text{ cal g}^{-1}$
3. Potassium abundance = 0.1%
4. ^{40}K abundance 4,600 Myr ago = 0.151%
5. ^{40}K half-life = $1.25 \times 10^9 \text{ yr}$
6. Heat of ^{40}K decay = 0.8 MeV
7. Melting point of the Moon = $1,500 \text{ } ^\circ\text{C}$
8. Total heat to melt the Moon = 455 cal g^{-1} ($1.9 \times 10^{10} \text{ erg g}^{-1}$)
Initial heat rate = $3.94 \times 10^{-7} \text{ cal g}^{-1} \text{ yr}^{-1}$
Compute time to melt, t :

$$455 = 3194 \times 10^{-7} \int_0^t \exp(-0.693 t / 1.25 \times 10^9) dt$$

where $t = 1,800 \text{ Myr}$

Table 2 Other radio-isotopes, half lives, and effectiveness

Radio isotope	Estimated Initial Abundance (No. of atoms per g of MgSiO ₃)	Half life (Myr)	Absorbable* disintegration energy (MeV)	Effectiveness† relative to ⁴⁰ K
¹²⁹ I	3 × 10 ¹⁴	17	0.075	0.09
²⁴⁴ Pu	0.2 × 10 ¹⁴	76	15	0.26
²⁶ Al	1.5 × 10 ²¹	0.74	1.6	2.2 × 10 ⁸
⁶⁰ Fe	6 × 10 ¹⁹	0.3	3	4.0 × 10 ⁷
²³⁸ U	2 × 10 ¹⁴	4.5 × 10 ³	48.3	0.96
²³⁵ U	2 × 10 ¹⁴	0.72 × 10 ³	43.3	
⁴⁰ K	2.3 × 10 ¹⁶	1.25 × 10 ³	0.8	1

Thus after 10 Myr, ⁶⁰Fe is negligible and ²⁶Al is down to the ⁴⁰K level.

* Neutrinos escape.

† Initial heating rate relative to ⁴⁰K.

are all indicative of, or consistent with, the existence of a basalt-rich shell, the source of the mare lavas. Thus the moon must have melted to a much greater depth to have produced this differentiation than it would have if the differentiated shell were only anorthosite rock (Ca Al₂ SiO₈ plus other low-melting substances) which is only 60 km thick on the near side of the Moon.

The strongest evidence for a completely molten early moon is provided by the lunar gravitational and magnetic data. It used to be generally accepted that there was no iron core in the moon; its mean density, 3.34, is that of olivine. The existence of an iron core was first suggested in a theory of the non-hydrostatic shape of the moon, proposing that it is dynamically maintained by its finite strength³⁰. The two-cell pattern of convection necessary to explain the shape seems compatible only with the existence of a core radius between 0.1 and 0.3% of the lunar radius³⁰. More recently, evidence for the existence of an iron core has come from the extensive remanent magnetism found in the lunar surface rocks. Most of the samples of basalt and high-grade breccia possess a stable, natural remanent magnetism, and the magnetic anomalies mapped³¹ by orbiting satellites are consistent with the laboratory findings. The directions of the magnetisation of the lunar crust must be such that no dipole field outside the moon exists, for an exceedingly small limit to this has recently been set by the Apollo 15 and 16 subsatellite magnetometer measurements³². This fact can be interpreted on the theory that the lunar shell was magnetised by an internal field³³. Although other explanations for an internal field have been suggested, the dynamo mechanism in which fluid motions in a liquid iron core are responsible seems to be the only tenable one. Three lines of evidence, none as yet conclusive, are compatible with an iron core of 400–500 km radius existing in the moon. This data suggesting a conducting or dense core is obtained from electromagnetic induction^{34,35}, from seismology²⁸, and from the moment of inertia factor²⁹, respectively. Tozer³⁶ has rediscussed the accretion process and concludes that gravitational energy can be released in the deep interior by shock waves from impacts of accreting material. The argument concerning this energy source has assumed that the accreting material falls to the growing moon from infinite distance. Instead, Ransford³⁷ has set up realistic models in which the moon accretes from material orbiting around the Sun at about 1 AU and from material thrown into it from other parts of the solar nebula by perturbations. He concludes, using experimental data on the effectiveness of impacts of convection of less kinetic energy into heat during impacts, that only a fraction of the outer 50 km of the moon would have melted through gravitational energy.

The magnetic maps of the far side of the moon show large anomalies over the deep basins; thus it seems likely that parts of the anorthosite highland shell have magnetisations greater by an order of magnitude than those of the mare basaltic flows³⁸. This suggests that the lunar magnetic field was present a few hundred Myr after the lunar origin and existed until at least 3,200 Myr ago, after which, at an unknown time, the

dynamo action ceased. Recent studies³⁹ of the palaeointensity of this field suggest that it was about 1 G at 4,000 Myr ago and had diminished to about 0.03 G at 3,200 Myr ago.

Thermodynamic arguments on the heat required to maintain a dynamo generating such a strong field from such a small core show that more powerful heat sources were present than those which could have been due to U, Th, and ⁴⁰K, assuming their concentrations to be the same as in the chondritic meteorites. The energy to maintain a molten core and drive the palaeodynamo had to be supplied quickly or else solid state creep in the silicate mantle rather than conduction would have been the dominant thermal transport process and would have prevented the internal temperature from reaching the melting point⁴⁰. The convecting core is a heat engine in which the 'useful' work done is the Joule heat dissipated by the electric currents in it. The efficiency of this dynamo cannot be greater than the fractional difference between the temperature at the centre and outside of the core multiplied by a factor equal to 2/5 for a uniformly distributed heat source⁴¹. From the adiabatic gradient in the core, this efficiency can be calculated, it is 0.016. A minimum value for the heat dissipated by toroidal currents to give the palaeofield can be estimated, it is 4.8 × 10⁹ W at 4,000 Myr ago (near that in the Earth today⁴²). Assuming, as a minimum, that the Joule heat dissipated in generating the toroidal field is twice that given above, the minimum heat generated in the core 4,000 Myr ago was 10¹² W⁴³.

Radio-elements and speculative transuranics

Table 1 gives the time of melting the Moon from ⁴⁰K, assuming no heat loss and 0.1% abundance. Other radio-isotopes, their

Fig. 1 Barrier penetration Factor B against α -particle energy in MeV.

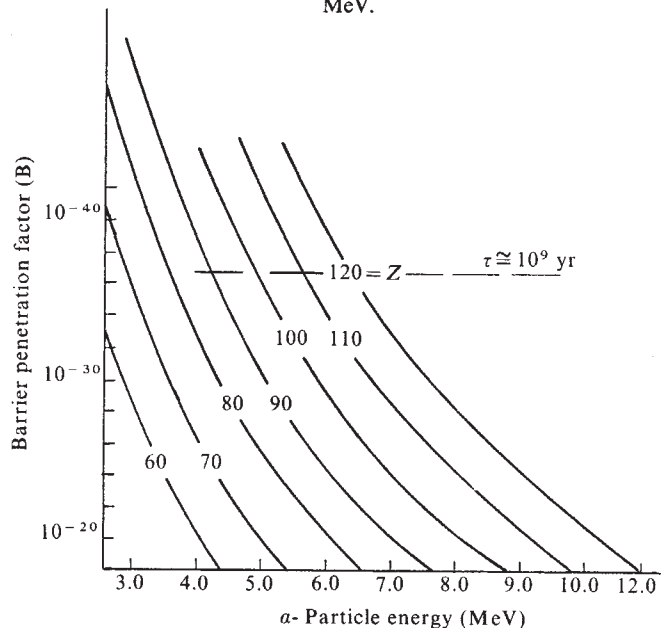


Table 3 Quantum numbers of the heavier elements				
$n + 1$	n	1	Element or Z	Likely chemistry*
8	5	3	Ac, Th, Pa, U, Np, Pu, Am, Cm, Bk, Cf, Ei, Fm, Md. 102	{ Actinides, similar to the lanthanum group
		6	(103), (104), (105), (106), (107), (108), (109), (110), (111), (112)	
		7	(113), (114), (115), (116), (117), (118)	
	8	0	(119), (120)	119 should be an alkali metal and 120 an alkaline earth
9	5	4	(121), (122), (123), (124), (125), (126), (127), (128), (129), (130), (131), (132), (133), (134), (135), (136), (137), (138)	{ Similar to actinium and lanthanum

* Elements which should be soluble in iron are $Z = 108, 109, 110$, and possibly $Z = 114, 115, 116$.

half lives, and their effectiveness relative to ^{40}K are listed in Table 2. We conclude that radio-element heating in the Moon by ^{40}K , ^{26}Al , and $^{235,238}\text{U}$ was sufficient to melt the Moon and thus produce a molten core, but on a time scale an order of magnitude too long, also none of these elements are soluble in iron and so cannot power a dynamo. The heat contribution from the short-lived (0.3 Myr) ^{60}Fe (ref. 44) was 50% complete in the first 0.3 Myr and was sufficient to melt the core if the Moon had formed early enough⁴⁵, and the same applies to ^{26}Al , evidence for the existence of which in the early solar system has recently been discovered⁴⁶. But most dynamic calculations⁴⁷ show that the time of formation of lunar sized objects by accretion from the solar nebula is about 100 Myr and it is probable that a similar time elapsed between the end of nucleogenesis and the formation of the nebula by contraction from an interstellar dust cloud⁴⁸. ^{244}Pu might be the source if its abundance were substantially higher than the estimate made here⁴⁹, but it too is not soluble in iron.

Next we turn our attention to potential heating by the transuranics. Supposing elements of $114 \leq Z \leq 127$ exist, what would be their chemical properties? These are indicated in Table 3, bearing in mind that no one has yet examined experimentally the chemistry of the elements filling the g valence electrons. The assignment of the transuranics to an order of filling of the s, p, d, f, and g electron shells (Table 3) allows us to predict that the elements which should be soluble in the iron core of the planets and moons have $Z = 108, 109, 110$, and

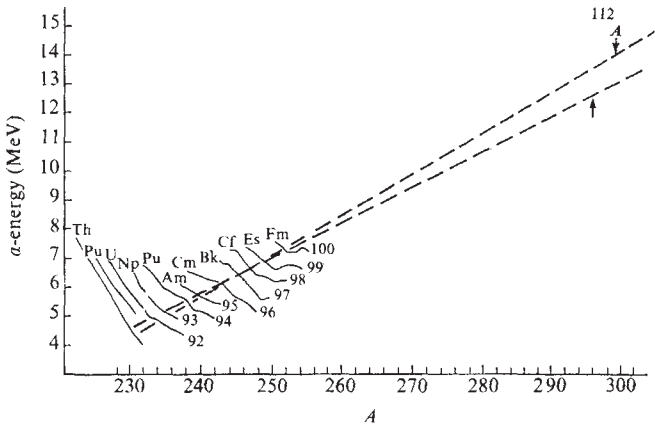


Fig. 2 Energy of α emission against atomic number A .

possibly 114, 115, and 116. If any of these have lifetimes sufficiently long, then we may have found a heat source soluble in iron which might, therefore, be important for producing an early magnetic field.

What can be said about the stability of elements $114 \leq Z \leq 128$ against α -particle emission? Straight forward application of the Gamow-Gurney-Condon theory of α emission by quantum mechanical tunnelling yields the systematics for the barrier

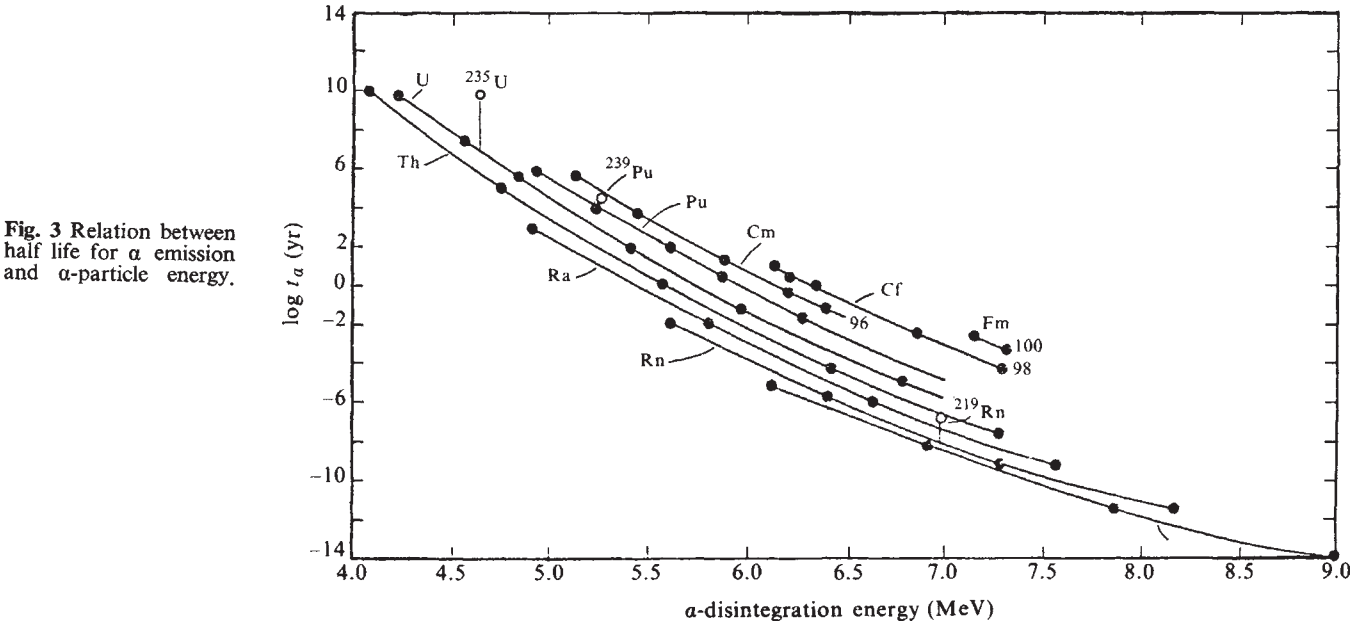


Fig. 3 Relation between half life for α emission and α -particle energy.

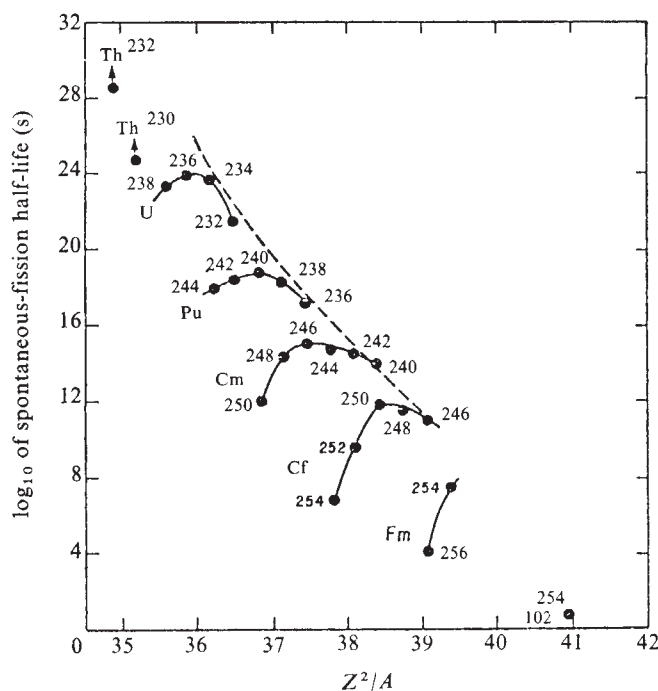
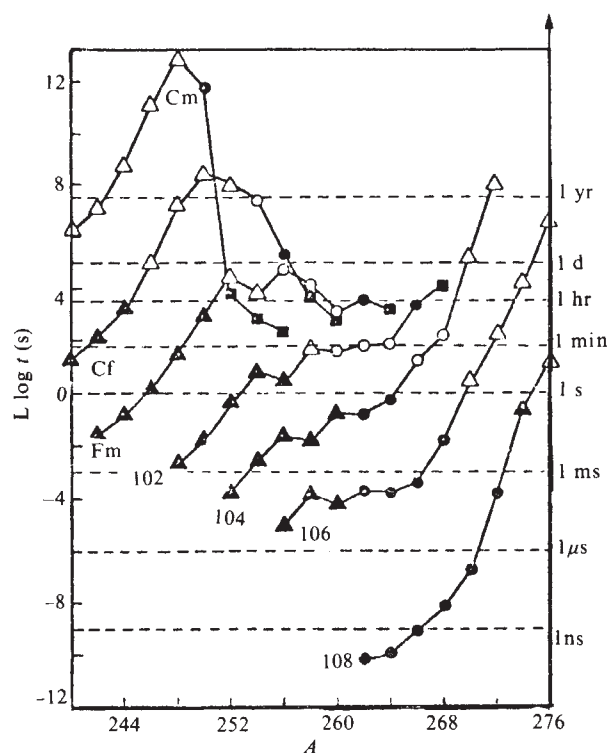


Fig. 4 Relation between half life for spontaneous fission and Z^2/A .

penetration factor B shown in Fig. 1 (adapted from ref. 50). The lifetime of $Z \sim 114$, for example, must be at least 1,000 Myr. Assuming the α -particle velocity to be $\sim 10^9 \text{ cm s}^{-1}$ inside a nucleus of diameter $\sim 10^{-12} \text{ cm}$, the number of collisions against the Coulomb barrier is $\sim 10^{21} \text{ s}^{-1}$, so to obtain a lifetime of $\sim 1,000 \text{ Myr}$ requires a barrier factor of, $B = (10^9 \times 3 \times 10^7) (10^{21}) = 3 \times 10^{37}$.

According to Fig. 1, for $B = 3 \times 10^{37}$ and $Z \sim 114$, the α -particle energy must not exceed about 5.7 MeV. Then the giant haloes, of diameters indicating α energies up to 14 MeV, must have been caused by α decay of the daughters of the trans-

Fig. 5 Relation between total half life and A .



uranics still existing, but not by the elements of 10^2 – 10^3 Myr lifetimes themselves.

Let us consider the α -particle systematics of the known transuranics. The known α -particle energies are plotted against A in Fig. 2 (adapted from ref. 51). For elements $90 \sim Z \sim 100$, the trend is for increasing α energy with increasing Z , with an indicated α energy of 11–12 MeV for element $^{114}284$. Therefore, the trend is for even shorter lifetimes with increasing Z as shown in Fig. 3 (ref. 51). The same is true of decay by spontaneous fission, see Fig. 4 (refs 51 and 52). How could the theoreticians^{53–55} reverse these trends and so account for lifetimes of $\sim 10^2$ – 10^3 Myr by α decay? They must keep the α energy below 5.7 MeV. The α energy is given by (for example, element $^{114}284$) the following expression for conservation of energy.

$$E = M(^{114}284) - M(^{112}280) - M(\alpha)$$

If a theoretical method can be devised to decrease the mass of the parent and increase the mass of the daughter nucleus, then E can be decreased below the required limit of 5.7 MeV. In practice, energies of nuclear cores are computed for shapes varying from spherical through a sequence of prolate and hour-glass deformations. For each shape there is a corresponding nuclear potential from which single particle wave functions and their energies are evaluated using the Schrödinger equation. The sum of the core energy and the energies for the single particle wave functions give $M(^zA)$. By using nuclear potentials giving small values of $M(^zA) - M(^{z-2}A-4)$, the theoreticians^{53–55} have been able to keep α energies below 5.7 MeV and thereby account for lifetimes as long as 1,000 Myr against α decay. In the same *ad hoc* way, prolate and hour-glass potentials for the ground state account for sufficiently long lifetimes against decay by spontaneous fission^{52–56}. The trends of lifetime obtained by this kind of postulation are shown against neutron number and mass (see ref. 22).

A recent calculation of lifetimes of superheavy elements has been made by Möller and Nix⁵⁶ adding to energies computed from a liquid drop model, fine corrections from shell model calculations of energies of single particle states in various potential energy functions. They find for $Z = 126$, $N = 228$ a lifetime too short (20 yr) for it to be found in nature. To calculate a lifetime 1,000 Myr, major changes have to be made in the nuclear model, for example, making the nucleus toroidal. For elements of $Z \sim 115$ small changes in the theory allow lifetimes to be sufficiently long. (A review of the state of the theory has been given by Hodgson⁵⁷).

In short, the Moon could be melted by ^{40}K and U in the first 2,000 Myr, but iron soluble heat sources seem to require the long-lived transuranics. ^{60}Fe could melt the Moon only if it assembled very early in less than 20 Myr and could not generate the magnetic field. We estimate that about 10^{-5} – $10^{-7} \text{ J g}^{-1} \text{ yr}^{-1}$ are required to keep the dynamo going. This would extend the effectiveness of ^{60}Fe to about 8.3 Myr. Ray and Kohman⁴⁴ estimates a factor of uncertainty of 3 in the ^{60}Fe half life. So this number might be as long as 25 Myr, far short of the required time.

Conclusion

There is an acute problem in obtaining, using conventional ideas, strong enough heat sources to melt the Moon 4,400 Myr ago and to generate its magnetic field in an iron core. We conclude that long-lived radioactive isotopes were present in the early Moon, although of their existence there is as yet little evidence. We consider that the elements of the 'island of stability' could have melted the early Moon and, as they may be soluble in iron, in driving the core dynamo. If so they must have half lives of 100–1,000 Myr. If they exist, many aspects of the origin of the solar system will require rethinking.

That these fundamental questions have emerged in the study of the Moon shows the key importance of the primaevial record that it has retained in its crust, which is much more complete than in any other planet, and also of the returned samples from

the Apollo and Luna missions have made possible radioactive age determinations and palaeomagnetic studies. This work reinforces the case for further fundamental studies of the Moon⁵⁸.

Received 8 June; accepted 4 November 1977.

1. Urey, H. C. *The Planets, Their Origin and Development* (Yale University Press, New Haven, 1952).
2. Runcorn, S. K. *Phil. Trans.* **258**, 228 (1965).
3. Sonnett, C. P., Colbourne, D. S. & Schwartz, K. *Icarus* **24**, 231–255 (1975).
4. Reynolds, J. H. *J. geophys. Res.* **68**, 2939–2956 (1963).
5. Brown, G. M., Eglinton, G., Runcorn, S. K. & Urey, H. C. (eds), *The Moon: A New Appraisal from Space Experiments and Laboratory Analyses* (The Royal Society, London, 1977).
6. Taylor, S. R. *Lunar Science: A Post-Apollo View* (Pergamon, Oxford, 1975).
7. Runcorn, S. K. *et al. Proc. R. Soc. A* **325**, 157–174 (1971).
8. Collinson, D. W., Stephenson, A. & Runcorn, S. K. *Phil. Trans. R. Soc. A* **285**, 241–247 (1977).
9. Rutherford, E., Chadwick, J. & Ellis, C. D. *Radiations from radioactive substances* (Cambridge University Press, Cambridge, 1950).
10. Gentry, R. V. *Science* **169**, 670–673 (1970).
11. Gentry, R. V. *et al. Phys. Rev. Lett.* **37**, 11–14 (1976).
12. Stephan, C., Epherry, M., Cieslak, E. & Sowinsky, M. *Phys. Rev. Lett.* **37**, 1534–1536 (1976).
13. Jelley, N. *et al. Nature* **265**, 35–36 (1977).
14. Kettle, B. H., O'Kelley, G. D., Stoughton, R. W. & Halperin, J. *Phys. Rev. Lett.* **37**, 1734–1737 (1976).
15. Sparks, C. J., Raman, S., Yakel, H. L., Gentry, R. V. & Krause, M. O. *Phys. Rev. Lett.* **38**, 205–208 (1977).
16. Cahill, T. A. *et al. Phys. Rev.* (in the press).
17. von Wimmersperg, U. & Sellschop, J. P. F. *Phys. Rev. Lett.* **38**, 886–888 (1977).
18. Hodgson, P. *New Scientist*, **74**, 706 (1977).
19. Larimer, J. W. & Anders, E. *Science* **175**, 981–982 (1972).
20. Flynn, G. J. & Loubet, M. *Nature* **268**, 717–718 (1977).
21. Wood, J. A., Dickey, J. S., Jr, Marvin, V. B. & Powell, B. N. *Geochim. cosmochim. Acta Suppl.* **1**, 965–988 (1970).
22. Hubbard, N. J. & Minear, J. W. *Geochim. cosmochim. Acta Suppl.* **6**, 1057–1085 (1975).
23. Papanastassiou, D. A. & Wasserburg, G. J. *Geochim. cosmochim. Acta Suppl.* **6**, 1467–1490 (1975).
24. Lugmair, G. W., Scheinin, N. B. & Marti, K. *Geochim. cosmochim. Acta Suppl.* **1**, 1419–1429 (1975).
25. Urey, H. C., Marti, K., Hawkins, J. W. & Lir, M. R. *Geochim. cosmochim. Acta Suppl.* **1**, 965–988 (1971).
26. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **7**, 3221–3228 (1976).
27. Sonett, C. P. & Duba, A. *Nature* **258**, 118–121 (1975).
28. Nakamura, Y. *et al. Geophys. Res. Lett.* **1**, 137–140 (1974).
29. Blakeshear, W. T. & Capcynski, J. P. *J. geophys. Res.* **82**, 1699–1701 (1977).
30. Runcorn, S. K. *Proc. R. Soc. A* **296**, 270–284 (1967).
31. Coleman, P. J. & Russell, C. T. *Phil. Trans. A* **285**, 489–506 (1977).
32. Russell, C. T., Coleman, P. J. & Schubert, G. *Science* **186**, 825–826 (1974).
33. Runcorn, S. K. *Phys. Earth Planet. Int.* **10**, 327–335 (1975).
34. Goldstein, B., Phillips, R. S. & Russell, C. T. *Geophys. Res. Lett.* **3**, 289–292 (1976).
35. Sonnett, C. P. & Wiskerchen, M. J. *Geochim. cosmochim. Acta Suppl.* **8**, (1977).
36. Tozer, D. C. *The Origin of the Solar System* (ed. Dermott, S.) (in the press).
37. Ransford, C. A. *Lunar Science Abstracts VIII* 793–794 (1977).
38. Lin, R., Anderson, K. A., Bush, R., McGuire, R. E. & McCoy, J. E. *Geochim. cosmochim. Acta Suppl.* **7**, 2691–2703 (1976).
39. Stephenson, A., Collinson, D. W. & Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **5**, 2859–2871 (1974).
40. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **6**, 2943–2953 (1975).
41. Backus, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1555–1558 (1975).
42. Gubbins, D. *Geophys. J.* **47**, 19–39 (1976).
43. Runcorn, S. K. *Science* (in the press).
44. Ray, J. C. & Kohman, T. P. *Canad. J. Phys.* **35**, 649–655 (1957).
45. Mizutani, H., Matsui, J. & Takeuchi, H. *The Moon* **4**, 476–489 (1972).
46. Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 109–112 (1976).
47. Kaula, W. M. & Harris, A. *Icarus* **24**, 516–524 (1975).
48. Runcorn, S. K. *Geochim. cosmochim. Acta Suppl.* **8**, 463–469 (1977).
49. Lugmair, G. W. & Marti, K. *Earth planet. Sci. Lett.* **35**, 273–284 (1977).
50. Enge, H. A. *Introduction to Nuclear Physics* 290 (Addison-Wesley, Reading, Mass, 1966).
51. Friedlander, G., Kennedy, J. W. & Miller, J. M. *Nuclear and Radiochemistry* 2nd ed. 229 (Wiley, New York, 1955).
52. Viola, V. E. Jr & Seaborg, G. T. *J. inorg. Chem.* **28**, 741–761 (1966).
53. Nix, J. R. A. *Rev. Nucl. Sci.* **22**, 65–120 (1972).
54. Fiser, E. O. & Nix, J. R. *Nucl. Phys. A* **193**, 647–671 (1972).
55. Möller, P. & Nix, J. R. *Phys. Rev. Lett.* **37**, 1461–1464 (1976).
56. Seaborg, G. T. & Bloom, J. L. *Sci. Am.* **220**, 57–67 (1969).
57. Hodgson, P. *New Scientist*, **75**, 152–153 (1977).
58. Runcorn, S. K. & Coleman, P. J. *Nature* **265**, 197–199 (1977).

Granulite xenoliths from Lesotho kimberlites and the lower continental crust

N. W. Rogers

University of London Reactor Centre, Silwood Park, Sunninghill, Ascot, Berkshire, UK

Six clinopyroxene–almandine granulite facies xenoliths have been analysed for major, trace and rare earth elements. Preliminary microprobe analyses suggest an origin near the base of the crust at temperatures of 660–830 °C and pressures > 9 kbar. Major element analyses indicate minimal chemical variation from a basaltic composition while the trace and rare earth element variation may be attributed to either magmatic or metasomatic activity.

INFORMATION about the composition of the upper mantle has been obtained from studies of ultra-basic xenoliths brought up by kimberlites. In contrast, relatively little work has been carried out on the crustal xenoliths, particularly in defining deep seated varieties. The crustal xenolith suite in Lesotho kimberlites includes sediments and regional metamorphic rocks, comparable with those exposed in basement complexes, as well as basic garnet clinopyroxene granulites rarely seen in the basement. The granulite xenoliths are thought to represent fragments of the deep crust on account of their high metamorphic grade and their ubiquitous though sparse occurrence in Lesotho kimberlites, and diatremes elsewhere, for example, Uganda (von Knorring and du Bois¹). This report describes a preliminary investigation into the chemistry of six such granulite xenoliths from Lesotho and comments on the bearing of these results on the evolution of the lower crust. The xenoliths are from the kimberlites at Matsoku (PHN 1646, 2852 and OVKE10303), Mothae (PHN1919) and Liqhobong (PHN2588).

Petrography

The xenoliths possess a distinctive equigranular, granoblastic texture and a limited mineralogy of clinopyroxene, garnet, plagioclase with accessory rutile and mica. The proportions of the minerals vary between samples but are generally in the range of 20–40% each of garnet and clinopyroxene with plagioclase constituting most of the remainder. Optical examination and preliminary microprobe analyses have shown the clinopyroxene to be a jadeitic diopside and the garnet a member of the pyrope–almandine series. Dark green kelyphytic rims around the garnets can attain thicknesses of up to 0.5 mm but are not as regularly developed as those surrounding garnets in ultra-basic xenoliths.

Rutile is the most abundant accessory mineral occurring as discrete grains randomly scattered throughout the rock. Mica, when present, is invariably in the process of decomposition to iron ore set in an aphanitic matrix. Its optical properties suggest that it is an iron rich biotite.

Petrographically, the xenoliths are members of the clinopyroxene–almandine granulite sub-facies² or high pressure granulite facies³, although the clinopyroxene and garnet compositions do possess some eclogitic affinities. Thus the assemblages must have crystallised under conditions of high temperature and high confining pressure.

Conditions of xenolith equilibration

As clinopyroxene and garnet are the only ferromagnesian minerals present in the xenoliths, the temperature of crystallisation can be estimated by the application of the geothermometer of Raheim and Green⁴. This is based on the partition

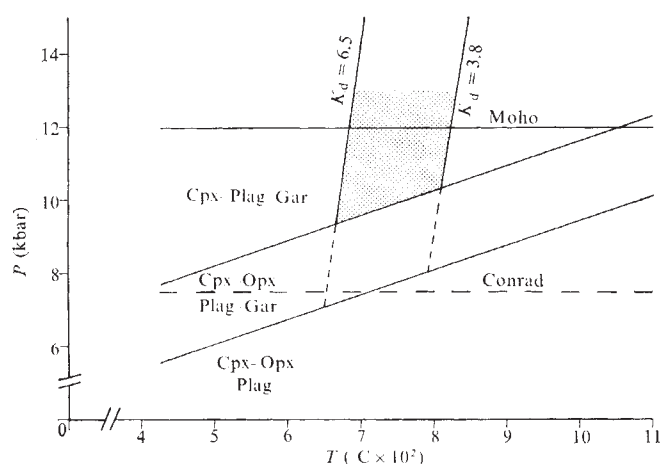


Fig. 1 Pressure, temperature diagram showing isopleths for the range of K_d values derived from the garnet-clinopyroxene (Gar-Cpx) pairs, superimposed upon the relevant phase boundaries for R698 Plag., plagioclase, Opx, orthopyroxene. The pressures corresponding to the depths of the Moho and Conrad discontinuities are shown.

of magnesium and iron between coexisting garnet and clinopyroxene expressed as a distribution coefficient, K_d . Values of K_d for five mineral pairs have been calculated from preliminary microprobe analyses, after an empirical correction for ferric iron⁶. The K_d values fall in the range 3.8–6.5, the isopleths corresponding to these two limiting values are shown in Fig. 1.

The pressure of equilibrium is more difficult to define, the sodium analysis of the pyroxenes is not precise enough to allow the calculation of the molecular proportion of the jadeite end member, previously used as a barometer⁶. Experiments by Irving⁷ on pyroxenite and granulite xenoliths of similar compo-

sition allows the definition of approximate pressure limits by extrapolating the observed phase boundaries to the temperatures derived from the K_d values of the Lesotho xenoliths. It should be emphasised that the pressure estimates are approximations due to the necessarily large extrapolation of the phase boundary from the original data. The experimental runs on a two-pyroxene granulite defined the boundary above which orthopyroxene is unstable, reacting with plagioclase to form garnet and clinopyroxene. By linear extrapolation, this boundary can be used to define the lower limit of equilibration of the Lesotho xenoliths, the latter being of similar composition to the xenolith studied experimentally (see Table 1). The upper pressure limit can only be roughly estimated, as the boundary above which plagioclase is unstable was not defined. Irving's results did, however, reveal a gradual decrease in modal plagioclase with increasing pressure. Although this effect cannot be extrapolated quantitatively to lower temperatures, it seems likely that the same phenomenon would occur in the less extreme conditions. As the Lesotho xenoliths contain relatively high modal abundances of plagioclase (20–40%), it is probable that they equilibrated at pressures not greatly in excess of the minimum pressure defined above.

By application of these constraints, it can be seen (Fig. 1) that the xenoliths probably equilibrated at pressure of 9–13 kbar at temperatures of 660–830 °C. The pressure estimates agree well with those expected at the base of the crust from seismic investigations (22–36 km)⁸. These figures are for the East Transvaal, there being no corresponding figures for Lesotho. The temperature estimates, however, range to high values compared with those expected for cratonic regions. This may be a true reflection of lower crustal conditions or a result of analytical error in the determination of sodium in the pyroxenes, affecting the calculated Fe(III)/Fe(II) ratio and, consequently, the K_d value.

It is of interest that two-pyroxene, garnet granulite xenoliths of similar bulk composition to the orthopyroxene free varieties have been found in Lesotho kimberlites⁹. According to ex-

Table 1 Major and trace element analyses of seven xenoliths

	PHN1646	PHN1919	PHN2852	OVKF10303	PHN2588	PHN2533	R698
SiO ₂	45.59%	45.26%	47.05%	49.25%	51.24%	50.39%	49.76%
TiO ₂	1.64	1.15	1.01	0.35	0.54	0.70	0.69
Al ₂ O ₃	14.40	16.42	17.88	17.44	16.69	15.14	16.83
Fe ₂ O ₃ *	14.25	11.53	1.47	9.62	10.58	12.16	1.86
FeO			7.71				7.09
MnO	0.21	0.18	0.16	0.15	0.18	0.18	0.17
MgO	9.78	9.86	7.52	10.26	8.60	7.02	8.25
CaO	11.55	8.23	9.59	11.11	8.47	9.05	11.07
Na ₂ O	1.65	4.99	3.85	1.86	2.86	3.47	2.78
K ₂ O	0.88	0.40	0.93	0.72	1.50	2.08	0.32
P ₂ O ₅	0.21	0.10	0.19	0.05	0.05	0.08	0.08
Total	100.14	98.12	99.96†	100.82	100.70	100.27	100.14‡
Ba	3,800 p.p.m.	520 p.p.m.	1,800 p.p.m.	500 p.p.m.	1,700 p.p.m.	3,700 p.p.m.	
Hf	3.4	0.8	0.7	—	—	—	
Nb	13	4	5	4	2	4	
Rb	25	14	12	8	23	31	
Sr	1,050	195	860	1,250	625	700	
Ta	0.7	0.1	0.1	—	—	—	
Y	27	13	7	5	4	10	
Zr	103	26	24	20	21	13	
Ce	36.7	13.8	7.0	3.9	—	—	ND
Nd	23.6	8.1	6.2	2.8	—	—	ND
Sm	5.6	2.3	1.6	0.8	0.6	—	ND
Eu	1.9	1.2	1.2	0.6	0.7	—	ND
Gd	6.6	2.4	—	—	—	—	ND
Tb	1.0	0.4	0.3	0.2	0.1	—	ND
Tm	0.4	0.2	—	0.2	0.1	—	ND
Yb	3.0	1.3	0.9	0.8	0.7	—	ND
Lu	0.5	0.2	0.2	0.2	0.1	—	ND
K/Rb	292	237	650	747	541	557	
Ba/Sr	3.6	2.7	2.1	0.4	2.7	5.3	

Major and trace element whole rock analyses of garnet pyroxene granulite xenoliths from Lesotho Kimberlites. R698, a two pyroxene granulite from Delegate is included for comparison. ND, not determined; —, not detected.

*Total iron as Fe₂O₃ (unless FeO is listed).

†Includes H₂O⁺ 2.36%, H₂O⁻ 0.24%. Analysis by O. von Knorring and D. T. Richardson.

‡Includes H₂O⁺ 1.10%, H₂O⁻ 0.14% from ref. 8.

periment, these xenoliths probably equilibrated at lower pressures than the orthopyroxene-free granulites, thus supporting the conclusion that the latter type are derived from near the base of the crust.

Major and trace element geochemistry

The major element analyses of six xenoliths are given in Table 1 together with trace and rare earth element (REE) data. Also included is an analysis of R698, the two pyroxene granulite used in the experiment⁷. The REE, Ta and Hf were determined by instrumental neutron activation techniques, similar to those described by Gordon *et al.*¹⁰; the remaining trace elements and the major elements were analysed by X-ray fluorescence.

A notable feature of the major element analyses is their restricted basaltic range, which in terms of alkali and alumina content, is comparable with high alumina and alkali basalts of island arc regimes¹¹. The alkaline nature of the xenoliths is a reflection of their high sodium rather than their high potassium contents, although the latter is not particularly depleted. The basaltic composition might suggest that the xenoliths represent pockets of magma that were trapped and solidified at lower crustal depths during a previous magmatic event; an origin that has been suggested for similar xenoliths elsewhere (as at Delegate⁷). The predominantly tholeiitic nature of earlier South African magmatism, however, suggests that this is unlikely as it is difficult to envisage how trapped tholeiitic magma could develop an alkaline and aluminous chemistry.

The narrow basaltic range of the analyses is in marked contrast to the wide compositional variation of granulites of metamorphic terrain¹². This could be a true reflection of lower crustal chemistry or an artefact caused by the selection of a narrow range of xenolith type. The xenoliths were selected on the basis of texture and mineralogy and no silicic varieties were included as they were not of comparable metamorphic grade. Also the xenoliths are not particularly depleted in K_2O , a common feature of granulites of metamorphic terrain¹³⁻¹⁵.

The limited major element variation also contrasts with the results of the trace and REE analyses, the latter showing considerable and significant variation between xenoliths. The chondrite normalised abundance diagram (Fig. 2) reveals a variation from xenoliths with light REE enriched patterns and no Eu anomaly (PHN 1646) to those with flatter REE patterns and positive Eu anomalies. One xenolith PHN2588, is thought to possess a light REE depleted pattern as Ce and Nd were not detected. The REE patterns do not particularly resemble those previously observed in other high grade metamorphic rocks (for example, ref. 16). The incompatible elements, Nb, Zr, Hf and Ta, vary comparably with the light REE, being most abundant in PHN1646, less abundant in the intermediate xenoliths and present in undetected amounts in PHN2588. This coherent behaviour of many of the trace elements is only slightly reflected in the small major element variation, such as PHN1646, the xenolith most enriched in the light REE, has a higher TiO_2 , total iron (as Fe_2O_3), MnO and CaO and a lower Al_2O_3 content than the other xenoliths. With so few analyses, however, it is difficult to assess the significance of this variation.

Before considering the significance of the trace element variation further, it is important to assess the possibility of contamination of the xenoliths by the host kimberlites. Despite stringent precautions taken during sample preparation to avoid inclusion of any carbonated material a comparison of trace element abundances in kimberlite and the xenoliths reveals that selective contamination may have taken place.

Four of the xenoliths possess high K/Rb ratios, in the range 540-747, and are much higher than usual values for kimberlite (about 200)¹⁷. Such a large difference, together with the grouping of the xenolith values despite the variation in the absolute abundances of the two elements in the xenolith suite, suggests minimal contamination.

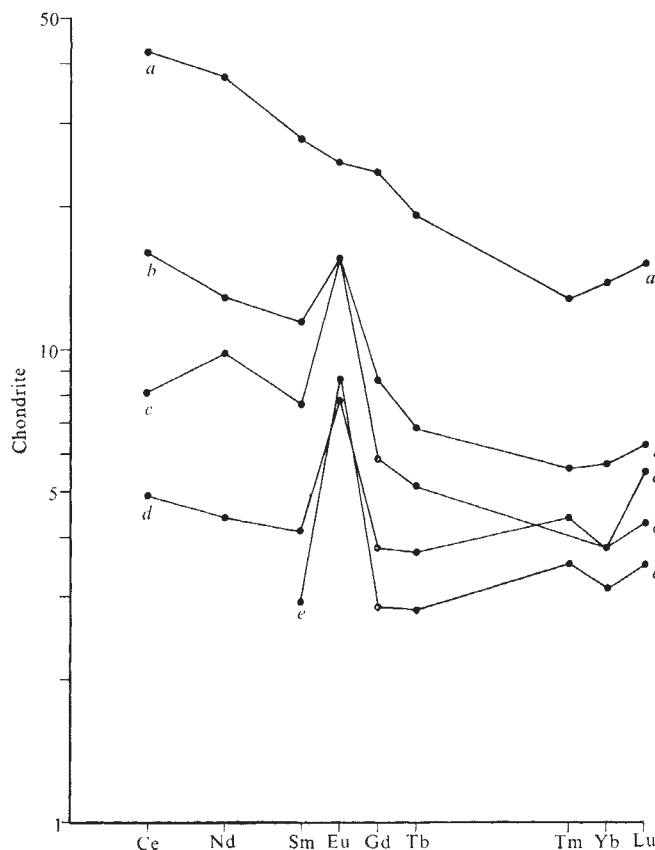
The effect of the kimberlite on the REE abundances of the xenoliths is thought to be minimal as the former rarely exhibit Eu anomalies, a common feature of the xenoliths. If the ob-

served REE variation was a result of contamination of uniformly depleted xenoliths, then it would be expected that the positive Eu anomaly should persist even in the sample most enriched with light REE. As this is not the case, PHN1646 showing no Eu anomaly, it seems unlikely that significant REE contamination has occurred. Similarly, as the other incompatible elements vary coherently with the REE, a lack of significant contamination can be inferred.

In contrast to these conclusions, the concentrations of Ba and Sr and the value of the Ba/Sr ratio each vary over an order of magnitude (see Table 1), generally corresponding to the kimberlite averages of 1,000 p.p.m., 700 p.p.m. and about 1.4 respectively. Also, the two elements behave in a way that is not comparable with the other trace elements. This apparently anomalous variation could be the result of contamination, the elements possibly having been mobilised during eruption by the volatile rich kimberlite.

The basaltic chemistry of the Lesotho granulite xenoliths, and that consequently inferred for the lower crust, contrasts with the observed, more silicic upper layers, in accord with theories suggesting a chemically zoned continental crust^{14, 15, 18}. From other experimental results¹⁹ the existence of a lower crustal layer with a less mafic composition than that inferred from the xenolith analyses has been proposed¹⁹. The less mafic composition was suggested as, in expected lower crust conditions, normal basaltic compositions take on a plagioclase-free eclogite mineralogy with a density too high to account for the observed seismic velocities. But, systems with high soda and alumina contents, comparable with the xenoliths, allow the stability of plagioclase at much higher pressures, in excess of expected lower crustal conditions. Thus the lower crust maintains a density below that of eclogite while possessing a mafic composition, although it is debatable how the lower crust

Fig. 2 Chondrite normalised relative REE abundance diagram for the five xenoliths analysed. a, PHN 1646; b, PHN1919; c, PHN2582; d, OVKF10303; e, PHN2588. Values for Gd in samples c, d and e are extrapolated, all other points refer to actual analyses.



developed this unusually sodic and aluminous chemistry originally.

Fyfe²⁰ suggested that crustal zonation resulted from intra-crustal partial fusion of rocks in the upper amphibolite or granulite facies followed by upward migration of the granitic partial melt leaving a relatively infusible and anhydrous residue of pyroxene granulite in the lower crust. In a similar model, Jakes and Taylor²¹ proposed that the residue would be plagioclase rich and thereby possess a positive Eu anomaly to balance the large negative anomaly seen in many Andean type igneous rocks that may be equated with Fyfe's granitic partial melt. Initially the xenoliths might seem to fit this model, those samples most depleted in the lithophile and rare earth elements possessing the most pronounced Eu anomalies. It is possible, however, for Eu anomalies to develop in plagioclase rich cumulates and gabbros (such as the Troodos gabbros²²). Therefore, such an anomaly cannot be regarded as unequivocal evidence for the previous occurrence of partial fusion and may indicate that the xenoliths were originally cumulates or gabbros and that the observed trace and rare earth element variation is the result of trapping different amounts of interstitial liquid in the original rock.

Two notable features of the trace element abundances concern the variation of K, Rb, Y and the heavy REE. Rb, frequently regarded as an incompatible element, does not vary with the other trace incompatible elements. Furthermore, Y and the heavy REE, elements easily accommodated by garnet and, therefore, in the context of garnet bearing assemblages, are compatible, show a similar, though less marked, depletion to the light REE Nb, Zr and so on. These features suggest the stability of a Rb bearing mineral (such as hornblende or biotite) in, and the absence of garnet from, the residuum during the solid-liquid fractionation. Feldspar is also thought to be a stable residual phase from the marked positive Eu anomalies present in all but one of the xenoliths. Hornblende and biotite are unusual minerals to crystallise directly from a basaltic liquid; olivine, plagioclase and pyroxene, are the most common phases to form initially. It, therefore, seems unlikely that the xenoliths are fragments of meta-gabbros or cumulates, but are most likely to be derived from the metamorphosed solid

residuum from the partial fusion of an amphibolite facies assemblage rich in feldspar, hornblende and/or biotite.

Finally, the possibility of metasomatic activity during the evolution of the granulites must be considered. The presence of accessory scapolite has been confirmed in similar granulite xenoliths from Lesotho⁸. This mineral, when found in granulite terrain, has been interpreted as having formed through metasomatic reactions and the addition of volatiles²³. Furthermore, of those xenoliths analysed, accessory mica is only found in samples PHN1646 and 1919, the two xenoliths most enriched in the light REE. Such an empirical relationship, however, must be further investigated before any firm conclusion can be made. Separate mineral and volatile element analysis should clarify this situation.

I thank Dr P. H. Nixon for supplying xenoliths and for helpful discussions. I also thank Dr O. von Knorring and Mr D. T. Richardson (University of Leeds) for analysis of sample PHN 2852, Dr I. L. Gibson (Bedford College, University of London) for providing facilities for neutron activation analysis and Dr P. Henderson for constructive criticism. This work was carried out while I was in receipt of a NERC advanced course studentship at the University of Leeds.

Received 9 March; accepted 21 July 1977.

1. von Knorring, O. & du Bois, C. G. B. *Nature* **192**, 1064–1065 (1961).
2. de Waard, D. *Am. J. Sci.* **263**, 455–461 (1965).
3. Green, D. H. & Ringwood, A. E. *Geochim. cosmochim. Acta* **31**, 767–833 (1967).
4. Raheim, A. & Green, D. H. *Contr. Miner. Petrol.* **48**, 179–203 (1974).
5. Ryburn, R. J., Raheim, A. & Green, D. H. *Lithos* **9**, 161–164 (1976).
6. Mysen, B. O. & Heier, K. S. *Contr. Miner. Petrol.* **36**, 73–94 (1972).
7. Irving, A. J. *J. Petrol.* **15**, 1–40 (1974).
8. Hales, A. L. & Sacks, I. S. *Geophys. J. R. astr. Soc.* **2**, 15–33 (1959).
9. Griffin, W. L., Carswell, D. A. & Nixon, P. H. in *Proc. 3rd Int. Kimberlite Conf.* 1977 (in the press).
10. Gordon, G. E., Randle, K., Goles, G. G., Corliss, J. B., Beeson, M. H., & Oxley S. S. *Geochim. cosmochim. Acta* **32**, 369–396 (1968).
11. Kuno, H. *Bull. Volc.* **29**, 195–222 (1966).
12. Clifford, T. N. *Spec. Pap. Geol. Soc. Am.* No. 156 (1974).
13. Heier, K. S. & Thorensen, K. *Geochim. cosmochim. Acta* **35**, 89–99 (1970).
14. Lambert, I. B. & Heier, K. S. *Geochim. cosmochim. Acta* **31**, 377–390 (1967).
15. Lambert, I. B. & Heier, K. S. *Lithos* **1**, 30–43 (1968).
16. Green, T. H., Brunfelt, A. O. & Heier, K. S. *Geochim. cosmochim. Acta* **36**, 241–257 (1972).
17. Harris, P. G. & Middlemost, E. A. K. *Lithos* **3**, 77–88 (1970).
18. Shaw, D. M. *Geochim. cosmochim. Acta* **32**, 573–602 (1968).
19. Green, T. H. *Phys. Earth Planet. Int.* **3**, 441–451 (1970).
20. Fyfe, W. S. *Geol. J. Spec. Issue No. 2*, 201–216 (1970).
21. Jakes, P. & Taylor, S. R. *Geochim. cosmochim. Acta* **38**, 739–745 (1974).
22. Kay, R. W. & Senechal, R. G. *J. geophys. Res.* **81**, 964–969 (1976).
23. Edwards, A. B. & Baker, G. J. *geol. Soc., Austr.* **1**, 1–32 (1953).

Geology and palaeontology of Neogene strata of Pakistan

David Pilbeam, John Barry & Grant E. Meyer

Peabody Museum, Yale University, New Haven, Connecticut 06520

S. M. Ibrahim Shah

Geological Survey of Pakistan, Quetta, Pakistan

M. H. L. Pickford & W. W. Bishop

Department of Geology, Queen Mary College, Mile End Road, London, UK

Herbert Thomas

Institut de Paléontologie, 8 rue de Buffon, Paris 75005, France

Louis L. Jacobs

Department of Geosciences, University of Arizona, Tucson, Arizona 85721

A joint study of the Potwar Plateau of Pakistan is yielding abundant material for stratigraphic and palaeontological reassessment.

THE Siwalik rocks of the Indo-Pakistan subcontinent have interested scholars for more than a century^{1,2}, in particular because of the presence in Siwalik beds of hominoid primates. *Ramapithecus punjabicus*, first described from India but known best from what are now quite large samples from Pakistan, is widely believed to be the earliest recognisable hominid or human

ancestor. At least three other hominoid species are known from the Siwaliks. An abundant associated vertebrate fauna is known from Siwalik rocks, spanning the time between 13 and 1 Myr ago. This period is believed to record the time of differentiation of Hominidae, and document the first radiation of higher primates into non-forest habitats. These rocks and their contained faunas figure widely in schemes of primate evolution, mammalian biostratigraphy and South Asian geology, and are increasingly important for the study of Asian climatic and tectonic history.

Since 1973, collaborative research between the Geological Survey of Pakistan (GSP) and the Peabody Museum of Yale University (YPM) has been in progress in the Potwar Plateau of

Pakistan. We give here a preliminary account of the stratigraphy and correlation of important areas within the Potwar Plateau. The results reported are tentative and subject to change because much detailed work remains to be done. Nonetheless we present them as working hypotheses to geologists, palaeontologists and palaeoanthropologists.

Potwar geology

The Potwar Plateau of the Punjab province ($72^{\circ}30' \text{ E}$, $33^{\circ}00' \text{ N}$) is an elevated area of some 20,000 km² bounded to the north by the Kala Chitta and Margala Hills, south by the Salt Range, east by the Jhelum River and west by the Indus River. Neogene molasse was deposited in subsiding basins on the southern flanks of the rising Himalayas. During and after deposition the area was subject to folding and faulting. A good deal of the Plateau is covered by late Pleistocene alluvium, but substantial amounts of Neogene rocks are exposed as badlands. Of particular importance, Siwalik sediments form the Soan synclinorium, the axis of which runs roughly east-west, and are also exposed in anticlinal and monoclinical belts along its northern and southern margins.

Siwalik rocks in the Potwar Plateau were first subdivided into 'Lower', 'Middle' and 'Upper' units by Pilgrim in 1910 (ref. 3). The subdivisions were then based on both lithological and palaeontological criteria, and clearly were meant to have temporal significance. Until recently, rock, faunal and temporal criteria and definitions have been entwined in discussions of Siwalik stratigraphy, causing confusion. In 1913 Pilgrim¹ subdivided the Middle Siwaliks into Dhok Pathan beds and included fauna, making the Dhok Pathan zone, and an underlying Nagri zone. The Lower Siwalik unit was described as the Chinji zone, while Tatrot and Pinjor zones were defined for the Upper Siwaliks. Pilgrim's 'zones' have something of the status of stages (time-rock units) of current stratigraphic usage. In theory, the units were intended to be bounded by isochronous surfaces. Lithology, however, was frequently used together with fauna in classification as though similar rock-type implied similar time. It is now known that because of facies change, gross lithology alone is a poor correlative tool; Pilgrim (ref. 3, page 186) noted this, although he often ignored its implications.

Anderson in 1927 used the terms 'Chinji stage', 'Middle Siwalik' and 'Upper Siwalik' solely in a lithological sense, and included discussions of thickness and facies variation. Essentially his concepts are 'formational' in current stratigraphic usage⁵; however, his 'Middle' and 'Upper Siwalik' categories are different from those of most other workers.

In the early 1930s Cotter and Colbert published slightly different stratigraphic schemes for the Siwaliks. Cotter⁶ used a 'stage' terminology ('Chinji Stage' and so on), the stages being defined on the basis of lithology and fauna. Colbert² subdivided the sequence into 'zones' using the same terminology, based almost exclusively on faunas. Lewis⁷ used a 1933 North American stratigraphic committee report in defining formations for the Siwalik subdivisions. Following Pilgrim, Anderson, Cotter and others he defined Tatrot, Dhok Pathan, Nagri, Chinji and Kamliak Formations, giving type localities and very brief lithological descriptions. But he also included descriptions of faunas as part of the definitions, and it seems that the units are stages of current usage, rather than formations. Gill⁸ used a 'stage' terminology for concepts that are basically formational.

In 1973 the Stratigraphic Committee of Pakistan⁹ formally defined formations solely on lithological criteria. The nomenclature used (Chinji, Nagri and so on) was that introduced by Pilgrim 60 yr earlier on the basis mainly of faunas, and which has since been used by many workers in various ways as rock and time-rock units. The use of these identical parallel terminologies has proved extremely confusing. We agree with Fatmi⁹ that Pilgrim's names should now be confined to properly defined lithological units. The formation names should not be used outside the type areas and 'mappably' contiguous areas. A new nomenclature for biostratigraphic and time-stratigraphic units is thus necessary.

Siwalik deposition is mainly fluvial and cyclic¹⁰⁻¹². Typical exposures are characterised by repetitive units, commencing with

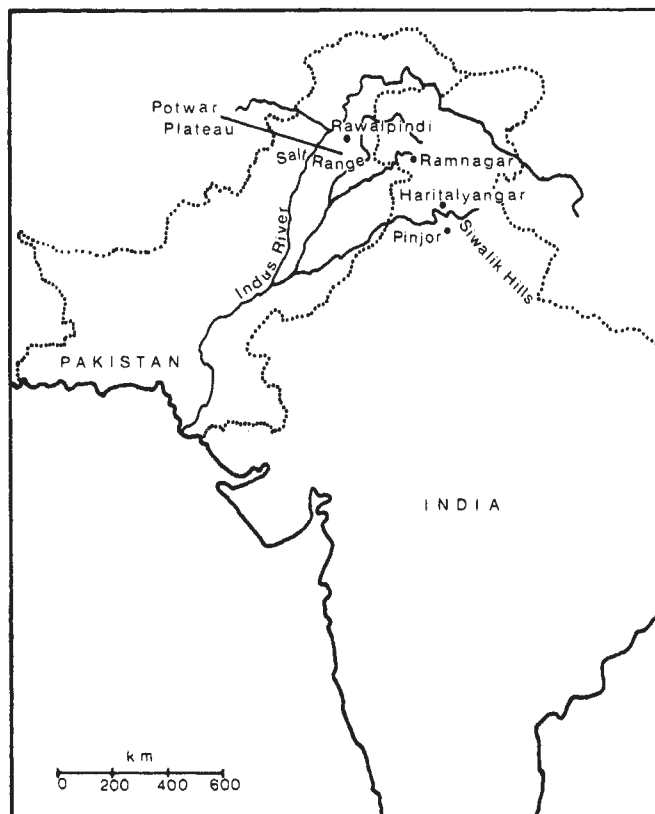


Fig. 1 Map of South Asia showing position of Potwar Plateau.

single sandstone bodies grading upwards into fine-grained silts and clays which are in turn abruptly truncated by the sandstone of the succeeding cycle¹⁰. The sandstones probably represent lateral accretion or point-bar, channel lag, cut and fill and channel splay deposits, the finer sediments being vertical accretion or overbank deposits which may preserve palaeosols. Other exposures are characterised by sequences almost lacking fine-grained sediments, while multistoried sandstone attain considerable thickness. As a whole, the beds imply a dominantly braided fluvial regime and a relatively rapidly aggrading multiple-channelled river.

The Chinji Formation ($72^{\circ}22' \text{ E}$, $32^{\circ}41' \text{ N}$) is defined⁹ south of Chinji Village on the southern margin of the Soan synclinorium, and consists of a series of sandstones separated by thick red silts. The succeeding Nagri Formation ($72^{\circ}30' \text{ E}$, $32^{\circ}46' \text{ N}$), defined north-east of Chinji Village near Sethi Nagri⁹, is made up of more massive sandstones with only minor overbank episodes.

Unfortunately the succeeding Dhok Pathan Formation⁹ is not defined in the same area as the Nagri Formation. Its type locality is at Dhok Pathan ($72^{\circ}31' \text{ E}$, $33^{\circ}8' \text{ N}$) on the northern flank of the Soan synclinorium, some 45 km north of Chinji Village and 40 km north and west of the Nagri type locality in the Gorge of the Gambhir River near Sethi Nagri. The Dhok Pathan Formation is, like the Chinji, predominantly argillaceous, although the overbank deposits of the former rarely match the rich red hues of the latter. Some nine fluvial cycles are recorded by us at Dhok Pathan village, where the formation is unconformably overlain by the much younger, horizontally bedded Potwar Silts.

We assume that the top of the Nagri Formation, as defined in the south in its type locality by transition from mainly arenaceous to mainly argillaceous beds, is time-equivalent to the similarly defined base of the Dhok Pathan Formation in the north. This may not be the case, however. It has been long realised, though much neglected, that these deposits are hardly atypical among fluvial sediments in showing significant lateral variation in thickness and lithology, often over quite short distances^{4,8,13}.

Detailed mapping and section measuring in these three stratotype areas should be completed within the next 2 yr. In addition

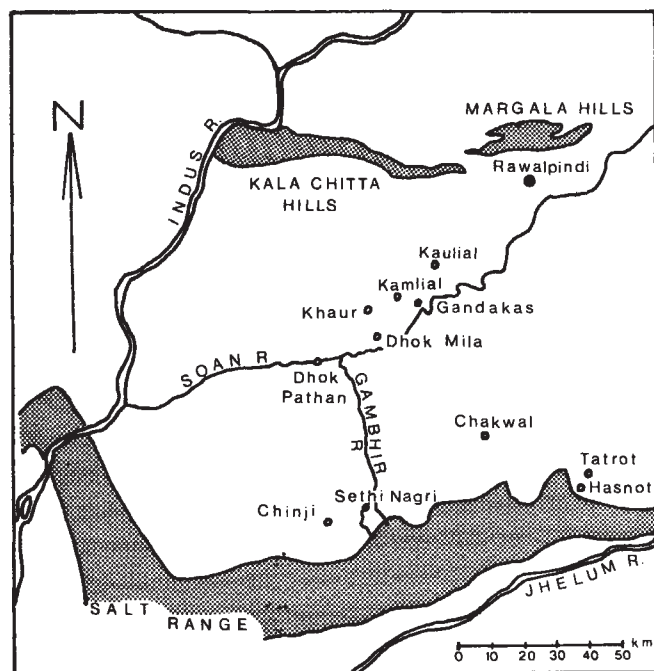


Fig. 2 Map of Potwar Plateau showing main fossil collecting areas.

we have explored in detail northern exposures to the east and north of Dhok Pathan and south of Khaur, near the villages of Dhok Mila, Dhok Ganja, Gandakas and Kaulial ($72^{\circ}30' E$ to $72^{\circ}45'$, $33^{\circ}9' N$ to $33^{\circ}22' N$). Major sandstone units have been strike-mapped north-east into this area from the Dhok Pathan type area, and it is clear that there are major lithofacies changes from west to east. Thus, as noted, at Dhok Pathan village, the Dhok Pathan Formation consists of some nine fluvial cycles, with overbank sediments dominant. The underlying beds presumed laterally equivalent to the Nagri Formation are arenaceous, consisting mainly of massive multistoried sandstones. Some 25 km to the north-east near Gandakas the same nine fluvial units are present in the Dhok Pathan Formation; however, near Gandakas the upper two-fifths of the underlying (presumed) Nagri Formation are argillaceous and lithologically similar to the Dhok Pathan Formation.

The Upper Siwalik Tatrot Formation⁷ is defined 85 km to the east of Chinji in a basin centred around the village of Hasnot ($73^{\circ}21' E$, $32^{\circ}52'30'' N$). The Tatrot Formation (not recognised by Fatmi) rests unconformably on sediments of presumed Middle and Lower Siwalik age. Although the formational names 'Dhok Pathan', 'Nagri' and 'Chinji' have been given to rocks in this area they have not yet been mapped into the type areas; caution should be exercised until this has been effected.

Mapping is to continue in each of these areas, in collaboration with an intensive programme to establish a regional magnetic polarity stratigraphy being undertaken by us and by a group from Peshawar University, Dartmouth College, the Lamont-Doherty Geological Observatory and the University of Arizona. Such studies, together with a continuing search for radiometrically datable sediments, must be completed before adequate temporal correlations between the various areas can be made. We assume that major sandstone units used in strike mapping (between, for example, the villages of Dhok Pathan and Gandakas) are minimally time-transgressive, at least for purposes of local correlation of middle and late Miocene sequences of strata.

Extensive faunas were collected in the late nineteenth and early twentieth centuries from the Potwar Plateau, principally from the Chinji, Dhok Pathan and Hasnot areas; a small fauna was also obtained from Sethi Nagri. These collections, together with those from Haritalyangar in India, 425 km to the south-east, and Ramnagar in Kashmir, 250 km to the east, formed the basis for

Table 1 Vertebrate faunas from the Chinji Formation

Mammalia	Artiodactyla
Primates	<i>Listriodon pentapotamiae</i>
<i>Sivapithecus sivalensis</i>	<i>Conohyus chinjiensis</i>
<i>S. indicus</i>	<i>Lophchoerus</i> sp.
<i>Ramapithecus punjabicus</i>	<i>Merycopotamus pusillus</i>
Creodonta	Tragulidae spp.
<i>Hyainailouros bugtiensis</i>	<i>Giraffokeryx</i> sp.
<i>Dissopsalis carnifex</i>	<i>Protragocerus gluten</i>
Carnivora	<i>Miotragocerus gradiens</i>
? <i>Viverra chinjiensis</i>	<i>Kubanotragus sokolovi</i>
Hyaenid indet.	? <i>Pseudotragus potwaricus</i>
<i>Percrocuta carnifex</i>	<i>Sivoreas eremita</i>
<i>Miohyaena cf. montadai</i>	<i>Gazella</i> sp.
' <i>Sivasmilus</i> '	Tubulidentata
(= <i>Paramachaerodus copei</i>)	<i>Orycteropus</i>
<i>Sivaelurus chinjiensis</i>	Rodentia
Felid indet. (very small)	cf. <i>Rhizomyidae</i> indet.
? <i>Sansanosmilus</i> sp.	<i>Copemys</i> sp.
<i>Martes lydekkeri</i>	<i>Megacricetodon</i> sp.
? <i>Martes</i> sp.	<i>Antemus chinjiensis</i>
<i>Vishnuonyx chinjiensis</i>	Reptilia
Mustelinae sp.	Chelonina
Amphicyoninae (large sp.)	<i>Trionyx</i> sp.
<i>Amphicyon</i> sp.	<i>Lissemys</i> sp.
<i>Vishnuocyon chinjiensis</i>	Ophidia
Perissodactyla	<i>Acrochordus</i> sp.
<i>Chalicotherium salinum</i>	Colubridae sp.
Rhinocerotidae spp.	Boidae indet.

the biostratigraphic zonation developed by Pilgrim and elaborated by many others^{1,2,7,14-20}.

The 'Chinji fauna' was based on material mainly from Chinji, and also on fossils from Ramnagar in Kashmir, from rocks of similar lithology (presumed at the time therefore to be of similar age). 'Nagri faunas' were partly based on the fossil mammals from Sethi Nagri, north-east of Chinji, but predominantly on specimens from sediments more than 400 km away at Haritalyangar with which they were correlated on very slender evidence. The bulk of the 'Dhok Pathan fauna' came not from Dhok Pathan itself but from around Hasnot, 90 km to the south-east on the opposite margin of the Soan synclinalorium. 'Tatrot faunas' came from Tatrot and Kotal Kund near Hasnot, and from Bhaun, 60 km to the west. Given that each 'fauna' is geographically heterogeneous, often markedly so, and that faunas from different areas were frequently 'lumped' on the basis of lithological criteria now known to be of dubious temporal value, one might well suspect that problems could develop; we aim here to clarify at least some of these.

The bulk of earlier collections from Chinji came mostly from the middle third of the Chinji Formation. Subsequently, collections by Brown, Lewis, Dehm and von Koenigswald, as well as the present project, sampled the upper parts of the formation. The Nagri type fauna comes from Nagri (presumably one of the villages of Sethi Nagri), north-east of Chinji. According to Pilgrim¹, the type locality for the faunal assemblage is 1,500 feet above the top of the Chinji Formation, some three-fifths of the way up the Nagri Formation in this area. What are probably very close or identical localities have been collected by Dehm, von Koenigswald and ourselves (GSP-YPM locality 311). At Dhok Pathan, we determined that the bulk of the classic fauna comes from the upper half of the formation, the lower portion of which is covered by the Soan River.

In the newly prospected regions to the east of Dhok Pathan we have collected fossils from over 30 fluvial cycles, nine of which we equate by strike mapping with the Dhok Pathan Formation at Dhok Pathan; the underlying cycles correlate with the upper part of the Nagri Formation, spanning approximately its top half. Within the Nagri Formation in the Dhok Mila-Gandakas-Kaulial area, we have informally designated the top few cycles the 'Utran unit', and the immediately underlying series, the 'Dhok Mila'. The upper four cycles of the Dhok Pathan Formation we have informally termed the 'Kundvali unit' while the lower five form the 'Gandakas unit'. On the basis of both relative stratigraphic position and close faunal similarity, we tentatively

Table 2 Vertebrate faunas from the Nagri Formation

Mammalia	<i>Merycopotamus nanus</i>
Insectivora	<i>M. dissimilis</i>
Soricidae indet.	<i>Dorcabune nagrii</i>
Primates	<i>Dorcatherium majus</i>
?Lorisidae indet.	<i>D. minus</i>
<i>Sivapithecus sivalensis</i>	cf. <i>Dorcatherium</i> sp.
<i>S. indicus</i>	cf. <i>Sivatherium</i> sp.
<i>Ramapithecus punjabicus</i>	<i>Gazella</i> sp.
cf. <i>Gigantopithecus</i> sp.	<i>Miotragocerus punjabicus</i>
Creodonta	<i>Selenoportax vexillarius</i>
cf. <i>Isohyaenodon</i>	? <i>Pseudotragus</i> sp.
Carnivora	<i>Boselaphini</i> very small n. gen., n. sp.
Viverrinae 2 sp.	Rodentia
?Herpestinae sp.	Sciuridae indet.
? <i>Progenetta</i> sp.	Gliridae indet.
<i>Palhyaena sivalensis</i>	<i>Rhizomyoides</i> sp.
? <i>Miohyaena</i> n. sp.	<i>Kanisamys sivalensis</i>
<i>Percrocuta carnifex</i>	<i>Progonomys</i> n. sp.
<i>Percrocuta grandis</i>	<i>Parapodemus</i> sp.
? <i>Sivaelurus</i> sp.	cf. ' <i>Mastomys</i> ' <i>colberti</i>
Machairodontinae	Reptilia
? <i>Martes</i> sp.	Chelonia
Mustelinae sp.	<i>Testudo</i> sp.
<i>Eomellivora</i> sp.	<i>Emydidae</i> sp. incl. <i>Kachuga</i> sp.
<i>Sivaonyx bathygnathus</i>	<i>Geomyda</i> sp.
<i>Amphicyon</i> sp.	<i>Trionyx</i> sp.
Proboscidea	<i>Lissemys</i> sp.
<i>Deinotherium</i> sp.	Crocodylia
Gomphotheriidae indet.	<i>Crocodylus palaeindicus</i>
Perissodactyla	<i>Gavialis hysudricus</i>
<i>Hipparion</i> small and large spp.	Ophidia
<i>Chalicotherium</i> cf. <i>salinum</i>	<i>Python</i> sp.
Artiodactyla	<i>Acrochordus</i> sp.
<i>Propotamochoerus hysudricus</i>	Colubridae indet.
<i>Propotamochoerus</i> sp.	Lacertilia
<i>Conohyus</i> sp.	<i>Varanus</i> sp.
<i>Tetraconodon</i> sp.	Amphibia
<i>Hippopotamodon sivalense</i>	Anura indet.
(= <i>Dicoryphochoerus titan</i>)	Mollusca
<i>Schizochoerus</i> sp.	Bivalvia
	Unionidae indet.
	Gastropoda indet.

correlate the southern Nagri faunas ('Nagri'/Sethi Nagri)/locality 311) with those from the middle of the Nagri Formation in the north.

Thus in the region of the stratotypes and 'mappably' adjacent areas we have collected faunal remains from most of the Chinji Formation, especially the middle and upper portions, and from a continuous sequence consisting of the upper half of the Nagri Formation and the entire Dhok Pathan Formation. These two faunal blocks are separated by a considerable stratigraphic, and probably temporal, gap which is only sparsely fossiliferous because of the predominantly arenaceous sedimentation of the lower half of the Nagri Formation in the type locality and adjacent areas.

New faunal analyses

About 13,000 catalogued specimens from approximately 350 localities have been recovered by the GSP-YPM expedition; these sample a diverse fauna of mammals, lower vertebrates and invertebrates. Most major groups are undergoing taxonomic revision. Previous collections in the British Museum (Natural History), Indian Museum, American Museum of Natural History, Yale Peabody Museum, Munich Geological Institute, and Geological Institute, University of Utrecht, are also being studied. In many cases it has been possible to determine quite closely the stratigraphic positions of previously collected specimens. Significant new material has been recovered, particularly primates, bovids, suids, carnivores and rodents (several hundred specimens), which should help considerably in biostratigraphic and other studies.

The fauna from the Chinji area contrasts markedly with that from Nagri and the area around Khaur; the latter, in contrast, apparently differs only minimally from that collected in the Dhok

Pathan Formation at Dhok Pathan and south of Khaur. The following faunal lists, based on our own collections with good stratigraphic control, or on previous material of known provenance, should be regarded as preliminary, although we feel that in broad outline this account is likely to stand. More detailed studies will follow as collections are expanded and major revisions are completed.

Chinji Formation

The vertebrate faunas (Table 1) have been collected from the upper two-thirds of the Chinji Formation in the type area (directly south of Chinji Village) and from some 10 km east and west of Chinji. Many other vertebrates and invertebrates have been collected from Chinji, and some have been recently revised²¹⁻²⁴. Others remain to be restudied.

Among several thousand specimens, we have collected neither cervids nor *Hipparion* from the formation. Rodents from surface collections reviewed by Black²⁵, with the possible exception of a rhizomyid, have also not been recovered by us. Overall faunal resemblances are to Astaracian²⁶ faunas of Europe and west Asia, and are especially marked among the suids, *Kubanotragus* (known elsewhere from Belometscheskaia in the Caucasus, Gabounia, ref. 27), and the cricetids, which resemble those from Europe. The murid is a new primitive species, possibly ancestral to later murids³⁶. There are a few resemblances to African faunas (Fort Ternan, Ngorora), presumably reflecting an earlier period of African-south Asian faunal connections, although they are not marked. An age of between 11 and 13 Myr is suggested for the Chinji Formation and its fossils based on biostratigraphic correlations to geochronologically controlled sequences elsewhere in the Old World²⁸.

Nagri Formation

The bulk of the mammals recovered by us (Table 2) come from the four fluvial cycles of the Dhok Mila unit south of Khaur, although some material is known from the uppermost Utran unit and from Sethi Nagri. At present, this is our best sampled unit. Other material remains to be studied and revised.

This fauna, the bulk of which comes from a relatively restricted stratigraphic range in the northern margin of the syncline, is clearly different from that of the Chinji area. This is to be expected considering the rather substantial thickness of Nagri Formation separating the two faunas; unfortunately this intervening section is only patchily fossiliferous. The fauna is most closely comparable with those from Eurasian rather than African sites. The bovids (especially *Miotragocerus punjabicus*), suids, rodents (*Progonomys* and *Parapodemus*) and the two species (compare ref. 29) of *Hipparion* suggest a correlation with late Vallesian or early Turolian faunas. The fauna from Samos, with an age of about 9 Myr (ref. 30), and Turkish faunas, aged between 9 and 10 Myr (ref. 31), are probably comparable in age with those from the upper part of the Nagri Formation.

Faunal resemblances, especially of suids and bovids, and preliminary geological mapping suggest that the southern Nagri Formation site(s) at Sethi Nagri sampled by Pilgrim, Dehm, von Koenigswald, and GSP-YPM locality 311 are very similar in age to those in the north.

The first appearance of *Hipparion*, as documented by us, falls in the lowest or 'Pari unit' (our informal usage) of the Nagri Formation. This suggests that much of the lower half of the Formation will prove to contain faunas of earlier Vallesian type.

Dhok Pathan Formation

An extensive fauna is known from the formation, both around Dhok Pathan Village and to the south and east of Khaur. Much of the earlier collected material has been located relative to our stratigraphic columns. The classic 'Dhok Pathan' fauna from the type area of the formation is known mainly from its upper half, informally termed the Kundvali unit by us. To the east, lower fossiliferous levels (Gandakas unit) have now been collected so that a continuous column has been sampled from sediments equivalent to the upper portions of the Nagri Formation through

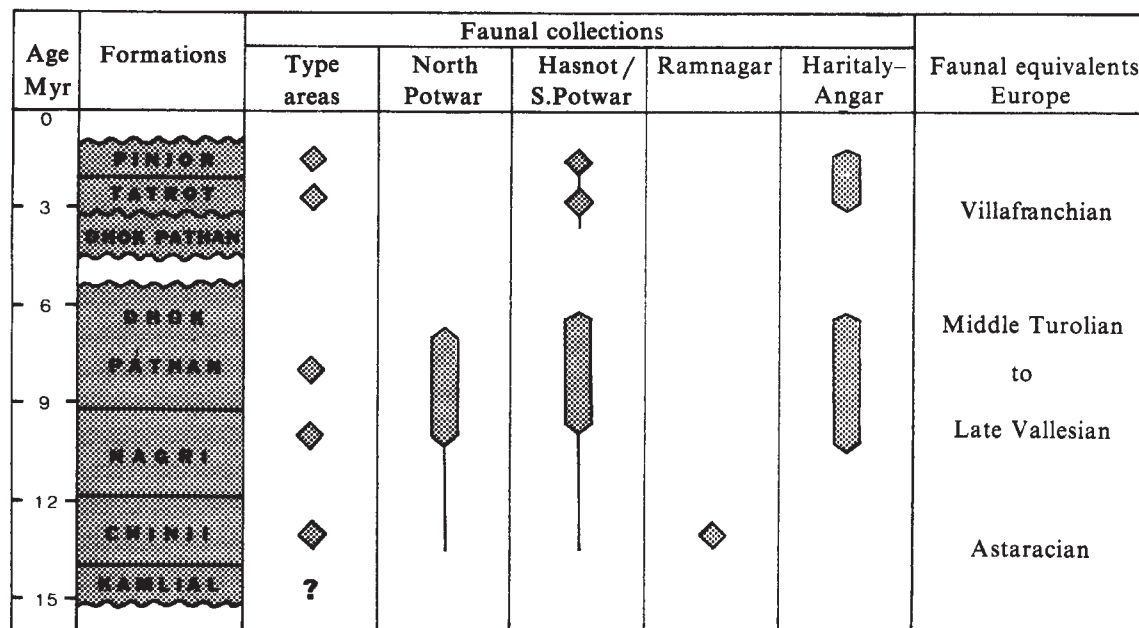


Fig. 3 Tentative stratigraphic chart showing Siwalik formations, main faunal collections and faunal comparisons.

to the upper levels of the Dhok Pathan Formation at Dhok Pathan.

Dhok Pathan Formation faunas are, for the most part, very similar to those of the underlying sediments; thus the suids, carnivores and bovids continue virtually unchanged. High in the Dhok Pathan Formation, however, between local sandstones 6 and 8 of our terminology, there are several interesting faunal changes. *Miotragocerus* and other bovids apparently become extinct; Reduncini make their first appearance (known elsewhere from Kenya in the Mpesida Beds and Lukeino Formation of the Baringo Basin dated at 7 and 6.5 Myr respectively³²), together with a species very similar to *Prostrepsiceros houtumschindleri* from Maragha in Iran, and a species of cf. *Sus*. Overall, the Dhok Pathan Formation faunas resemble those from the Turolian Land Mammal 'Age' as defined in Europe, North Africa and West Asia. A suggestion of absolute age can be obtained from radiometric dates on the Samos faunas of around 9 Myr (ref. 30), and of around 8 to 9 Myr (or perhaps less) on similar Turkish faunas³¹.

Of interest is the overall similarity between the faunas of the upper Nagri Formation and its equivalents and those from the Dhok Pathan Formation. Their probably rather restricted time range seems to fall close to the boundary between Vallesian and Turolian Land Mammal 'Ages'. Only at the top of the Dhok Pathan Formation is there evidence of some faunal change. It has long been assumed, however, that the 'Nagri' and 'Dhok Pathan' faunas were rather different, and it is worth considering why this view should have arisen, given the relative similarity of our collections from the type areas of the formations. As Pilgrim^{1,3} outlined, the 'Dhok Pathan fauna' contained specimens from around Dhok Pathan itself, as well as from Niki (or Nikki) and Hasnot. Hasnot, from which a substantial fauna is known, is in a relatively isolated part of the southern Potwar Plateau, 90 km from Dhok Pathan. A close inspection of the works of Pilgrim and in particular of Colbert² shows that, although a number of species are common to both the Dhok Pathan and Hasnot areas, most of the species of the 'Dhok Pathan fauna' that differ from those from the upper parts of the Nagri Formation both at Nagri and near Khaur, are in fact known mainly or exclusively from around Hasnot.

The supposed 'Middle Siwalik' deposits at Hasnot have yielded several species of Reduncini, cervids, several suids including *Sivachoerus*, *Hippohyus*, *Sivahyus* and *Sus*, the oldest Siwalik cercopithecoid *Presbytis sivalensis*, and a rodent fauna clearly distinct from that found in the upper part of the Nagri Formation and its equivalents.

Probably the deposits at Hasnot include a succession of rocks

of similar lithology to those at Dhok Pathan that is the same age as and younger than the type Dhok Pathan Formation. Rocks of similar age, so far unexplored, are probably present in the area east of Kaulial.

Potwar biochronology

The improper extension of formational names to lithological sequences that are not in mapped continuity with the type areas, especially in fluvial deposits where rapid changes in facies are common, cannot be too strongly condemned. Ultimately, we hope that correlations between the principal Potwar fossiliferous areas can be documented using non-faunal methods—strike-mapping, magnetic polarity stratigraphy and radiometric age determinations. Such efforts will be continued by both our group and that from Peshawar-Dartmouth-Lamont-Arizona. Until that time we believe that the biostratigraphic scheme for the Potwar Plateau outlined here will serve as a reasonable working hypothesis. Ultimately, an improved biostratigraphic zonation with names different from formational ones must be established.

Detailed lithostratigraphic and biostratigraphic work in the central Potwar region enables us tentatively to isolate two rock sequences (and time periods) for which faunas can be described. The earlier of these comes from the upper two-thirds of the Chinji Formation at its type locality, and most closely resembles Eurasian and African Astaracian and Astaracian-equivalent faunas, ranging in age from perhaps 11 or 12 to 13 or 14 Myr. The later faunal sequence spans the upper half of the Nagri Formation and equivalent rocks and almost the entire superposed Dhok Pathan Formation, comprising material from Nagri itself, Dhok Pathan and the region south and east of Khaur contiguous with the Dhok Pathan area. These faunas probably span the period between about 10 Myr and perhaps 7.5 or 8 Myr, on the basis of comparisons with other dated Eurasian and African faunas. Within this faunal sequence little change can be observed, although some (apparent) extinctions and appearances are recorded high in the Dhok Pathan Formation. A preliminary interpretation of faunas from the upper parts of the so-called Middle Siwaliks at Hasnot suggests that at least part of the fauna there is younger than the bulk of that from Dhok Pathan; an average age of around 7 Myr can be tentatively suggested for these younger elements.

The period between the Chinji Formation and upper parts of the Nagri Formation and equivalents is represented by poorly fossiliferous sandstones. Known faunas from these formations and equivalents are substantially different. Within the poorly

fossiliferous part of the column we locate the first appearance of *Hipparion*, in the basal 'Pari unit' of the Nagri Formation. Other workers have reported *Hipparion* from the Chinji Formation^{1,2,6}, but the provenance of these finds can be questioned^{3,3} or they consist of a very few isolated teeth which might well not have been *in situ*. In four seasons we have not located *Hipparion* in the Chinji Formation, and in our opinion this equid is an immigrant arriving after deposition of the Chinji Formation.

Other Siwalik faunas

Two other areas outside the Potwar Plateau yield abundant Miocene fossils, including primates—the regions around Ramnagar and Haritalyangar. Material from Ramnagar, 100 km north-east of Jammu in Kashmir, is apparently similar to faunas from the type Chinji Formation. Mammals include *Listriodon* and other characteristic species, while *Hipparion* is absent^{1,6}. Around Haritalyangar, Siwalik rocks are extensively exposed¹. The terms 'Chinji', 'Nagri' and so on have been widely used both for formations and faunas in these areas, in our view unjustifiably. At Haritalyangar the major Miocene fossiliferous horizons are exposed as a sequence of predominantly argillaceous beds in the 'Lower' and 'Upper Alternations'; these comprise almost 70 fluvial cycles^{11,12}. According to Pilgrim¹ and to recent work by teams from Chandigarh and Yale Universities, *Hipparion* is found throughout the section. But most of the fauna from the Haritalyangar region, including that used by Pilgrim to make up the bulk of his 'Nagri fauna', as well as that collected later by Lewis¹⁹, Prasad^{34,35} and the Chandigarh-Yale group, probably comes from a relatively restricted zone of less than 10 fluvial cycles, 20 or more cycles above the base of the Lower Alternations (with *Hipparion*). Recent analysis of faunas from Haritalyangar in Calcutta and at Yale (Lewis collections) by M.P., H.T. and L.L.J. suggests a close similarity of bovids, rodents and suids to those from the upper part of the Nagri Formation of the central Potwar Plateau. Such a correlation, however, needs to be confirmed independently, and is probably best done magneto-stratigraphically.

If correct these correlations suggest that the bulk of known Indo-Pakistan Lower and Middle Siwalik faunas fall into two groups: an earlier one (material from Chinji and Ramnagar) around 12 Myr old, and a younger one (Nagri, Dhok Pathan, Khaur, upper Hasnot, Haritalyangar) 10–7 Myr old. Some material from Khaur-Dhok Pathan, Hasnot and Haritalyangar probably fills the gap between the two groups, though only sparsely. This has important implications for faunal studies,

especially those of the hominoid primates, because some Lower Siwalik mammals may be almost twice as old as some from the Middle Siwaliks.

We have recovered almost 90 new hominoid primates during the past three seasons, mostly from the Khaur area. They are from a relatively restricted stratigraphic range, mostly from the Dhok Mila unit in the upper third of Nagri Formation equivalent strata. Some other material comes from Sethi Nagri and is probably of similar age, as estimated from faunal associations. The material seems to be sampled from at least three species, *Ramapithecus punjabicus*, *Sivapithecus indicus* and one similar to *Gigantopithecus*. Some of the specimens have relatively complete jaws, and also include the first hominoid postcranial remains from the Siwaliks. They are the subject of the following report.

This work was supported by the NSF, Smithsonian FCP, Government of Pakistan, Air France, CNRS, SRC and NERC. We thank the Director General of the GSP, Mr Mahmood Raza, Mr Hod French, Ms C. Badgley, Drs A. J. Gaston, E. Buffetaut, S. Wenz, J. Rage, F. de Broin, R. Hamilton, T. Hussain and M. Freneix, and the Ministry of Fuel, Power and Natural Resources of India.

Received 8 August; accepted 11 October 1977.

- Pilgrim, G. E. *Rec. Geol. Surv. India* **43**, 264 (1913).
- Colbert, E. H. *Trans. Am. Phil. Soc.* **27**, 1 (1935).
- Pilgrim, G. E. *Rec. Geol. Surv. India* **40**, 185 (1910).
- Anderson, R. V. *Bull. Geol. Soc. Am.* **38**, 665 (1927).
- Krumbein, W. C. & Sloss, L. L. *Stratigraphy and Sedimentation* (Freeman, New York, 1951).
- Cotter, G. *Mem. Geol. Surv. India* **55**, no. 2 (1933).
- Lewis, G. E. *Am. J. Sci.* **33**, 191 (1937).
- Gill, W. D. *Q. J. Geol. Soc., Lond.* **107**, 375 (1952).
- Fatmi, A. N. *Mem. Geol. Surv. Pakistan* **10**, 1 (1973).
- Duff, P., Hallam, A. & Walton, E. *Cyclic Sedimentation* (Elsevier, Amsterdam, 1967).
- Johnson, G. D. *Neogene Molasse Sedimentation in a Portion of the Punjab* (University Microfilms, Ann Arbor, 1971).
- Johnson, G. D. *Geol. Rundsch.* **66**, 192 (1977).
- Morris, T. O. *Q. J. Geol. Soc., Lond.* **94**, 385 (1938).
- Pilgrim, G. E. *Pal. Ind. NS* **8**, 1 (1926).
- Pilgrim, G. E. *Pal. Ind. NS* **18**, 1 (1932).
- Pilgrim, G. E. *Am. Mus. Novit.* **704** (1934).
- Pilgrim, G. E. *Mem. Geol. Surv. India* **26**, 1 (1939).
- Matthew, W. D. *Bull. Am. Mus. Nat. Hist.* **56**, 437 (1929).
- Lewis, G. E. dissertation, Yale Univ. (1937).
- Dehm, R. *et al. Abh. Bayer. Akad. Wiss.* **90**, 5 (1958).
- Dehm, R. *Abh. Bayer. Akad. Wiss.* **114**, 5 (1963).
- Heissig, K. *Abh. Bayer. Akad. Wiss.* **152**, 5 (1972).
- Hoffstetter, R. *Bull. Soc. Géol. France* **7**, ser. 6, 467 (1964).
- Modell, H. *Abh. Bayer. Akad. Wiss.* **135**, 5 (1969).
- Black, C. C. *Palaeontology* **15**, 238 (1972).
- Fahlsbuch, V. *Neurol. Stratigr.* **5**, 160 (1976).
- Gabounia, L. K. *Acad. Sci. Rep. Soc. Sov. Georgian. Inst. Palaeobiol.* 136 (1973).
- Berggren, W. A. & Van Couvering, J. *Palaeo. Palaeo. Palaeo.* **16**, 1 (1974).
- Hussain, S. T. *Abh. Bayer. Akad. Wiss.* **147**, 5 (1971).
- Van Couvering, J. & Miller, J. A. *Nature* **230**, 559 (1971).
- Sickenberg, O. *Geol. Jb.* **B15**, 1 (1975).
- Pickford, M. H. L. *Nature* **256**, 279 (1975).
- Simons, E. L., Pilbeam, D. & Boyer, S. J. *Nature* **229**, 408 (1971).
- Prasad, K. N. *J. Geol. Soc. India* **3**, 86 (1962).
- Prasad, K. N. *Palaeontology* **7**, 1 (1964).
- Jacobs, L. L. *Paleobios* **25** (1977).

New hominoid primates from the Siwaliks of Pakistan and their bearing on hominoid evolution

David Pilbeam, Grant E. Meyer, & Catherine Badgley

Departments of Anthropology and Geology and Geophysics and Peabody Museum of Natural History, Yale University, New Haven, Connecticut 06520

M. D. Rose

Section of Gross Anatomy, Department of Surgery, Yale Medical School, New Haven, Connecticut 06520

M. H. L. Pickford

Department of Geology, Queen Mary College, London, UK

A. K. Behrensmeyer

Division of Earth Sciences, University of California, Santa Cruz, California 95064

S. M. Ibrahim Shah

Geological Survey of Pakistan, Quetta, Pakistan

Siwalik deposits in the Punjab have yielded a rich collection of hominoid primate remains. Together with other recent finds they indicate the need for some changes in hominoid classification.

SINCE 1973 a team from the Geological Survey of Pakistan (GSP) and the Peabody Museum has collected fossils in the Potwar

Plateau, Punjab Province, Pakistan (72°30' E, 33°00' N) as part of a joint research project aimed at a better understanding of the geological, floral and faunal history of South Asia. Neogene rocks of the Siwalik Group are widely exposed in India and Pakistan as part of the South Asian alpine system. The Soan synclinalorium in the Potwar Plateau is the area in which all but one of the type localities for Siwalik formations and South Asian Land Mammal

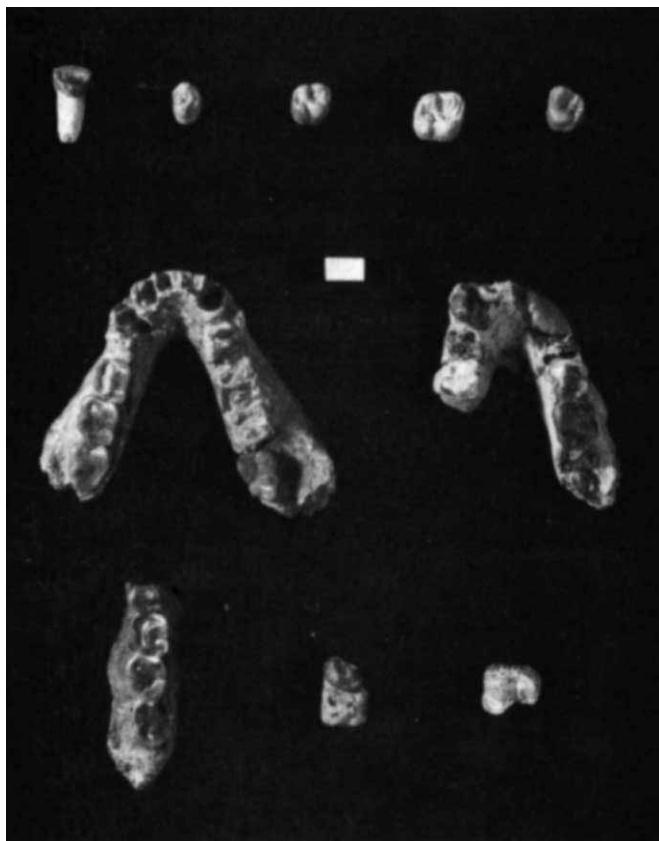


Fig. 1 Top row left to right: GSP 9903, 9906, 5019, 6206, 8702. Middle row: GSP 4622/4857, 9563/9902. Bottom row: GSP 6153, 7619, 7144.

Agcs are located (Figs 1 and 2 of preceding article). The lithostratigraphy and biostratigraphy of Siwalik deposits in the Plateau have been discussed in the preceding article¹.

More than 13,000 catalogued fossil specimens representing a diverse vertebrate and invertebrate fauna have been collected from more than 300 localities; 18 localities have yielded 86 new hominoid primate specimens representing a minimum of 43 individuals (Tables 1–3). The hominoids are accurately located stratigraphically with well documented information concerning lithological context and faunal associations. They approximately double the previously known hominoid collections². In addition, new stratigraphic information has facilitated further interpretation of earlier collections. Of particular interest are four localities (Table 2) in which remains of between five and nine hominoid individuals have been found together.

Detailed descriptions of the geology, revisions of major vertebrate and invertebrate groups, and more extensive descriptions and analyses of the hominoids will be published elsewhere as studies are completed. What follows is a preliminary announcement of the primate finds.

Geology and palaeoecology

Most of the new hominoid sites are along the northern rim of the Soan synclinorium between Dhok Mila and Kaulial. One, 311, is at Sethi Nagri, another, 38, south of Chinji; both are along the southern margin of the synclinorium. All the northern sites, with two exceptions (251 and 261), are situated stratigraphically in the upper third of the Nagri Formation; localities 251 and 261 are somewhat lower in the section. Locality 311 at Sethi Nagri is probably situated somewhat below the level of the bulk of the northern sites. Locality 38 is in the Chinji Formation.

Approximate ages can be assigned to the localities on the basis of faunal comparisons to dated sequences elsewhere in the Old World, and these are apparently supported by palaeomagnetic surveys which will be published soon. Locality 38 may be about 12 Myr old; 251 and 261 about 9.5–10 Myr old; and the rest of the

sites some 9.0 Myr old.

Abundant vertebrate and invertebrate faunal remains have been recovered from the Potwar Plateau both by us and by previous expeditions, and the biostratigraphic sequence is becoming clear. The upper Nagri levels are particularly well sampled, and new analyses of major taxonomic groups (rodents, carnivores, suids, bovids and equids) suggest close similarities to European and West Asian sites of late Vallesian and early Turolian age³.

Earlier studies of Siwalik lithology⁴, faunas^{5–7} and floras^{8,9} generated hypotheses about past habitats. These studies were large scale, involving the analysis of faunas from many localities and broad stratigraphic ranges, or used lithological or floral samples which could not be tied into firm stratigraphic or palaeogeographic frameworks. Only recently have more adequate studies started¹⁰. Our programme of detailed lithostratigraphical, magnetostratigraphical, sedimentological, taphonomical, faunal and floral analyses is aimed at a better understanding of Siwalik palaeogeography and past plant and animal associations.

Many primate localities are in pellet rock lithologies usually associated with minor grey-green sandstones within a red-bed sequence. In localities where several individuals are preserved, primates are often a relatively abundant component of the fauna. So far collections have been made in a taphonomically adequate way from few sites, nor have faunal analyses involving minimum numbers of individuals been completed. Possible faunal differences between primate and non-primate localities are being investigated. Preliminary faunal analyses from our 'best-collected' localities suggest that the overall mammal community lacked a diversity of large mammals and had low numbers of grazing species, herbivores above 100 kg and arboreal species. By comparison with recent and Pleistocene faunas associated with riverine environments, this suggests woodland or bush habitats with open patches of grassland rather than extensive forests. The sedimentary features and lithologies of the upper Nagri indicate a braided fluvial regime of low sinuosity rather than a meandering one, and a rapidly aggrading multiple-channelled river would

Fig. 2 Top row, left to right: GSP 11708, 11704, 9977/01/05/9564. Bottom row: 11536/7, 13163, 4230, 9977/01/05/9564.

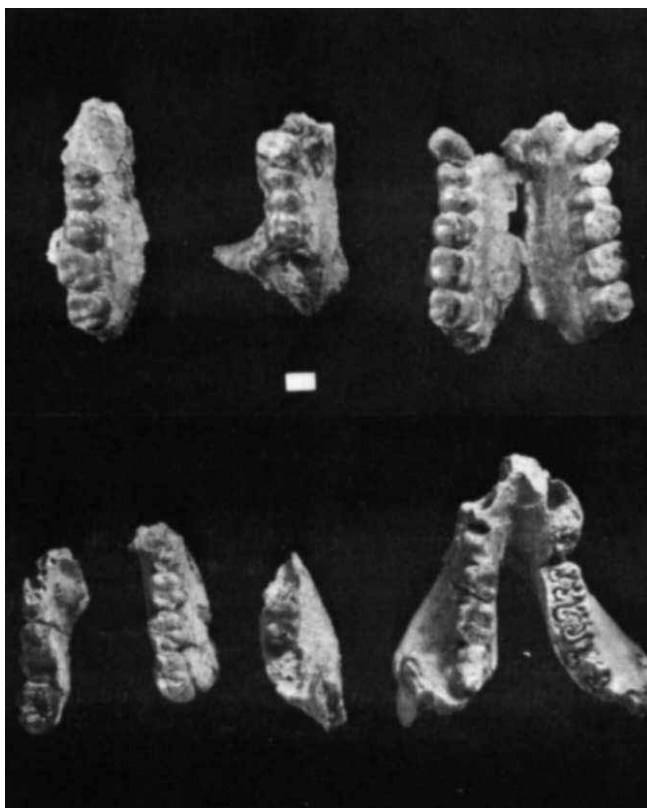


Table 1 Hominoid specimens collected from the Potwar Plateau, Punjab

Area	Locality	GSP no.	Specimen	Area	Locality	GSP no.	Specimen
Sethi Nagri	311	11536/7	Infant left mandibular corpus, dP ₃ , dP ₄ , associated M ₂	Khour	317	13166	Left P ₄
		10493	Left C			12647	Right M ¹
		11534	Left P ⁴ fragment			9972	Right M ²
		11999	Right M ¹			9896	Left M ²
		13162	Right M ¹ fragment			9969	Right M ²
		12000	Right M ¹ fragment			9895	Left M ³
		9986	Right M ¹			9900	Right M ³
		10500	Left M ² fragment			12709	Infant mandible, right C, dP ₃
		11533	Right M ² fragment			9563/9902	Mandible, left P ₃ , M ₁ , right M ₂ , M ₃
		7144	Left M ₁ fragment			13165	Right mandibular fragment, M ₁ - M ₃
		11998	Right M ₂			9565	Left C fragment
		13164	Central I			9899	Right M ₃
		12648	Central I			12654	Femoral fragments
		6663	Distal humeral epiphyseal fragment			9894	Femoral head fragment
		6664	Distal pollicial phalangeal fragment			13168	Distal phalangeal fragment
		6666	Distal phalangeal fragment			11708	Right maxilla, P ³ - M ³
		6454	Intermediate cuneiform			11786	Right maxillary fragment, P ³ - M ²
		12271	Distal humeral fragment			11704	Right maxillary fragment, I ² root - M ¹
		8928	Right I ¹			7618	Right M ²
		8925	Right C			11706	Right mandibular fragment
Khour	182	5019	Right M ¹	Khour	137	11707/85	Mandible, left M ₃
		5018	Left M ² fragment			7619	Left mandibular fragment, P ₃
		5067	Left M ³			9930	Left M ₃
		8702	Left M ³			7611	Radial diaphysis
		4622/4857	Mandible, left M ₁ - M ₃ , right M ₃			11867	Femoral head and neck
		4230	Right mandibular fragment with M ₂			3293	Left I ¹
		5464	Right I -			12568	Left C
		8679	Right C			5001	Right M ₂
		5712	Left P ₃ fragment			211	Right M ¹
		5020	Left P ₄			6758	Left M ³
		4635	Right M ₂			6759	Right M ₃
		4735	Right M ₃			224	Left mandibular fragment, P ₂ - M ₃
		8926	Right M ₃			7308	Right M ¹ fragment
		8927	Left M ₃			6178	Femoral head fragment
		4664	Left distal calcaneal fragment			6160	Right mandibular fragment, P ₃ - M ₃
Khour	260	9977/01/05/	Maxilla, left and right C, M ³	Chinji	38	6206	Right M ²
		9564	right I ² ; mandible, right C, left P ₄ - M ₃ ; right condyle; Cranial and facial fragments			13171	Left I ¹
		9897	Left maxillary fragment			6999	Right I ¹
		9903	Right I ¹			251	Right M ²
		9898	Left I ¹			261	Right P ⁴
		13167	Left C			309	Left C
		9906	Left P ⁴			1023	Left C fragment
						10785	Talar fragment
						11003	Left C
						780	Left C fragment

probably have been characterised by a mosaic of more and less open habitats rather than extensive riparian forest.

There have been too few palynological studies of Siwalik age sediments⁸⁻¹⁰ to indicate clearly either the range of habitats at a particular time or habitat changes through time. Available evidence suggests a change during the deposition of the Chinji and Nagri Formations from mainly subtropical, forest habitats to more open, less low-lying habitats. Schaller¹¹ has summarised views on the modern vegetation of the area: evergreen and semi-evergreen forests have quite restricted distributions, most habitats being deciduous forest or thorn forest. Although South Asian Neogene climates may have been warmer and with more precipitation than Recent climates, it is possible that evergreen forests (including gallery forests) were never very widespread during the time of deposition of Siwalik group rocks. As noted, such limited information as we have suggests non-evergreen forest contexts for at least some of the major primate localities.

Primates

There is an extensive primary literature on Siwalik primates, and they have been mentioned and discussed many times². Approximately 90 hominoid specimens representing more than 60 in-

Table 2 Tentative listing of minimum numbers of hominoid individuals

Locality	No. of specimens	Minimum nos individuals			
		G	R	Si	Total
182	17	-	4	2	6
260	21	-	4	5	9
311	19	1	5	5	6
317	10	-	2	3	5
137	1	-	-	1	1
191	1	-	-	1	1
207	1	-	-	1	1
211	1	-	-	1	1
221	2	-	1	-	1
224	3	-	2	-	2
226	1	-	2	-	1
227	2	-	2	-	1
230	1	-	-	1	1
251	1	-	1	-	1
261	1	-	-	1	1
309	2	-	-	2	2
310	1	-	2	-	1
314	1	-	-	1	1
38	1	-	-	1	1
19	86	1	17	25	43

G, *Gigantopithecus*; R, *Ramapithecus*; Si, *Sivapithecus indicus*.

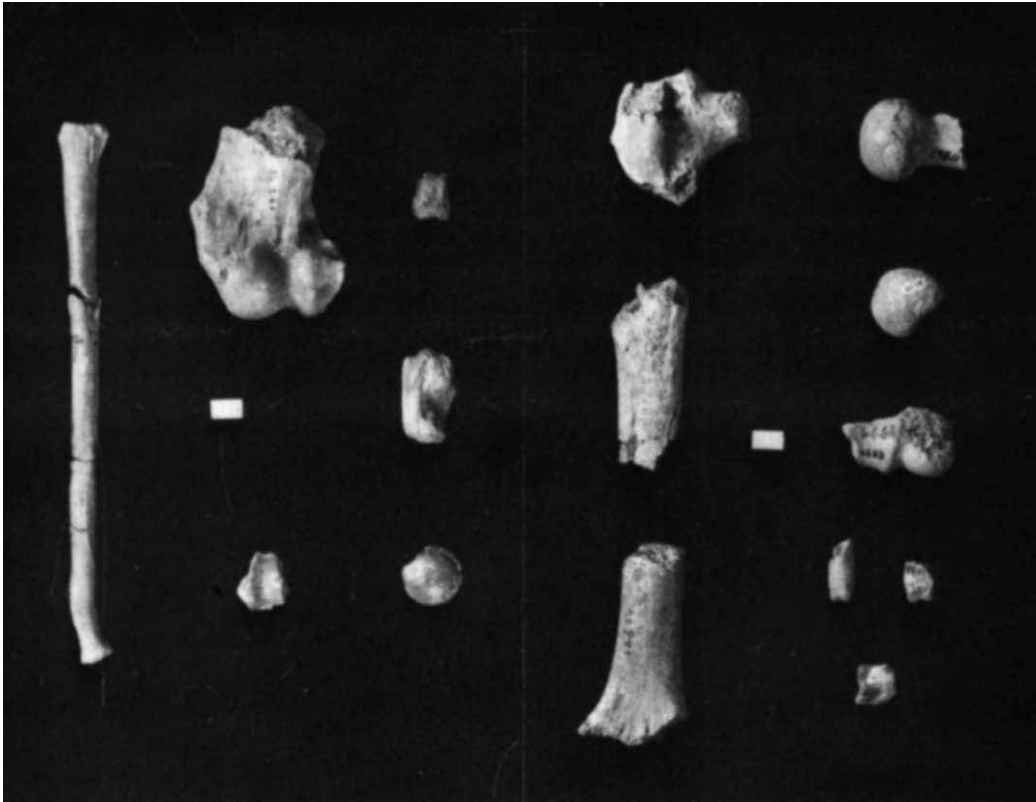


Fig. 3 Left to right, first column: GSP 7611; second column: top, GSP 12271, bottom, GSP 10785; third column: top, GSP 6664, middle, GSP 4664, bottom, GSP 9894; fourth column, GSP 12654; fifth column, top to bottom, GSP 11867, 6178, 6663, 6666 and 13168, 6454.

dividuals are known from previous collections. The Potwar Plateau yielded some isolated teeth and a few jaws, the bulk from the Chinji Formation at Chinji; several specimens came from around Hasnot, and a few individual teeth were known from other localities. Ramnagar in Kashmir was the source of a few hominoids, but the best collections came from Haritalyangar in India.

At least four hominoid species are represented in the Siwaliks, thought previously to range in age between about 14 Myr and 6 Myr. The four species are *Ramapithecus punjabicus*, *Sivapithecus sivalensis*, *S. indicus* and *Gigantopithecus bilaspurensis*. Until recently species of *Sivapithecus* have been classified in *Dryopithecus*, but it now seems preferable to separate them generically from *D. fontani* and other dryopithecines.

In several recent reviews of Miocene Hominoidea these Siwalik species and others from elsewhere in the Old World have been placed in either the Hominidae or Pongidae. *Ramapithecus* has often been included in Hominidae, while species of the other genera have usually been considered pongids¹². This arrangement has not been accepted universally.

The new specimens from Pakistan discussed here (Tables 1–3), together with much new material from other Old World localities, facilitate a different view of earlier Neogene hominoid classification and evolution.

The new hominoids are discussed here taxonomically. Assignment of some individuals to species is difficult or impossible, and the scheme outlined is provisional. More comprehensive descriptions and discussions are being prepared. Measurements of the more complete specimens are given in Tables 4 and 5.

At least one species of *Ramapithecus*, *R. punjabicus* (Fig. 1), is represented in Siwalik rocks; the genus is found in the Chinji and Nagri Formations, and possibly in rocks equivalent to the Dhok Pathan Formation. The probable age range is 13–8.5 Myr.

The new material from the Potwar Plateau helps considerably in understanding previous finds as well as adding significant new information. Particularly fine specimens are the adult mandibles GSP 4622/4857 from locality 182 and GSP 9562/9902 from

Fig. 4 Tentative stratigraphic-morphological distribution of large hominoid groups with possible phylogenies. P, Pongidae; D, Dryopithecidae; S, Sivapithecinae, R, Ramapithecinae; A, Australopithecinae; H, Homininae. (Sivapithecidae should be Ramapithecidae; note added in proof.)

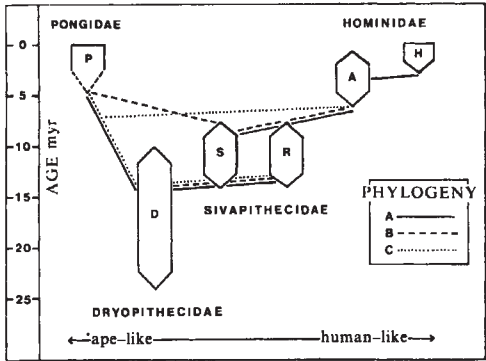


Table 3 Distribution of hominoid parts

Part preserved	Specimens
Cranial	
Associated maxillae and mandibles	1
Adult maxillae	4
Adult mandibles	9
Infant mandibles	2
Isolated teeth	
P ¹	6
C	8
P ⁴	4
M ¹	6
M ²	11
M ³	6
I ¹	3
C	2
P ³	1
P ²	1
M ¹	1
M ²	3
M ³	5
Postcranial	13

Table 4 Tooth measurements on hominoid maxillae

		9977/01		11704	11736	11708
		L	R	R	R	R
I ²	md	—	5.5			
	bl	—	7.5			
C	max	14.2	13.4	14.6		
	tr	10.8	10.8	10.7		
P ³	md	9.3	9.3	9.2	9.2	9.5
	bl	11.8	14.3	11.2	—	11.6
P ⁴	md	8.7	7.5	7.7	8.5	8.3
	bl	12.4	12.4	11.7	12.2	12.0
M ¹	md	11.5	12.1	11.1	12.0	12.4
	bl	13.3	13.3	12.8	—	13.1
M ²	md	13.5	13.6		12.5	13.7
	bl	14.6	14.2		13.4	14.0
M ³	md	12.7	12.7			12.5
	bl	13.7	13.5			13.8

locality 260, and an infant mandible, GSP 12709, also from locality 260. These specimens show that the incisor region was very narrow in *Ramapithecus*, canines small, anterior premolars and canines closely packed together, and postcanine tooth rows and mandibular corpora posteriorly divergent. Most teeth in the upper and lower dentitions are now known. Certain features are worth noting: P₃ has a small but distinct lingual cusp, and its long axis is oriented at some 45° to the mesio-distal line of the tooth row. Unworn cheek teeth resemble those of *Australopithecus* and *Homo* quite strongly, particularly the maxillary molars. Occlusal surfaces are constricted and there is marked buccal (mandibular teeth) and lingual (maxillary) flare in unworn specimens (compare ref. 13). Occlusal surfaces broaden with wear and can be almost flat and still show no dentine. This is because occlusal enamel is very thick (between 2.5 and 3.0 mm on mandibular buccal cusps), as shown in a few broken specimens (GSP 8926) and by comparison of unworn and worn homologues of similar size. Mandibular rami are relatively robust; symphysis have marked superior and inferior transverse tori.

Gigantopithecus bilaspurensis (Fig. 1) is based on a complete mandible from the Haritalyangar area. It is likely to have an age of around 8.5 Myr^{1,2,10}, and is not significantly younger than the other hominoid primates from that area.

One partial molar from locality 311, GSP 7144, is tentatively assigned to *Gigantopithecus*, as is a previously described molar, GSI D175 from Alipur near Hasnot¹⁴. Both are probably 9.0–10 Myr old. Occlusal morphology is rather similar to that of

Ramapithecus, and occlusal surface enamel is thick (about 3.5 mm on the mandibular buccal cusp of GSP 7144).

Specimens of *Sivapithecus indicus* (Fig. 2) are known from deposits ranging between about 13 and 8 Myr. Several relatively complete new specimens (especially GSP 9977/01/05/9564 from locality 260) facilitate mandibular and lower facial reconstructions. Tooth rows are subparallel with broad incisor regions and postcanine tooth rows that are markedly concave buccally.

Occlusal morphology in *S. indicus* resembles the other genera, although molars are somewhat broader and there are other minor differences. Occlusal surfaces are constricted on cheek teeth with marked buccal and lingual flare; enamel is thick (about 3 mm on mandibular buccal cusps).

Canines are projecting and moderately dimorphic and exhibit mesial, distal and apical wear; P₃s are closely approximated to the canines, their long axes rotated to lie about 45° to the mesio-distal line of the tooth row. Mandibular rami are deep; the symphysis is long with a prominent inferior transverse torus and a relatively small superior transverse torus.

S. sivalensis is the most enigmatic of the Siwalik hominoids and it is not absolutely clear that it exists as a separate species.

Parts of 13 hominoid postcranial bones (Fig. 3) have been collected so far and fit into three size groups. The largest specimen is a partial distal right humerus, GSP 12271, with the lateral supracondylar ridge, lateral epicondyle, capitulum and radial fossa preserved entire, and the coronoid and olecranon fossae and trochlear surface preserved in part. In its size and morphological features this specimen is similar to the distal humerus of adult female gorillas. Eight specimens are close in size to the corresponding bones of adult pygmy chimpanzees. This group includes a partial distal right humerus, GSP 6663, with parts of the capitular and trochlear surfaces preserved. Hindlimb specimens include a femoral head, GSP 6178, a femoral head plus part of the neck, GSP 11867, the proximal, mid-shaft, and distal non-articular parts of a right femur, GSP 12654, a left intermediate cuneiform, GSP 6454, and the distal end of a proximal hallux phalanx, GSP 6664. These specimens, together with two distal ends of phalangeal bones, GSP 6666 and GSP 13168, show some morphological similarities to extant hominoid species, although these are not as marked as in the case of the large specimen. Three fossils are smaller. One of them, a partial left radius, GSP 7611, is clearly juvenile. A partial right talus, GSP 10785, which includes most of the trochlear surface, the lateral process, and most of the posterior calcaneal articular surface, and a partial femoral head, GSP 9894, come from approximately adult macaque-sized animals. It is not certain that they are from adults. A partial calcaneus,

Table 5 Measurements of hominoid mandibles

		9564/9905	13165	4622/4857	4230	9563/9902	6153	6160
C	max	12.3						
	tr	10.1(R)						
P ₃	max					11.2		11.0
	tr					7.0		7.0
P ₄	md	* 9.4(L)					8.1	8.5
	bl	—				—	9.5	9.8
M ₁	md	*13.0	13.0	11.5		*10.5	10.3	11.1
	bl	*12.0(L)	*10.9	9.5		* 9.5	9.8	—
M ₂	md	14.5	14.0	12.7	14.7	12.7	12.4	12.7
	bl	12.7(L)	11.7	10.5	12.7	10.7	10.9	10.7
M ₃	md	*15.5	*14.5	12.9	—	—	12.3	13.7
	bl	—(L)	—	10.5	—	—	10.8	—
Breadth at C		*35		22.5		*25		
Breadth at M ₂		*47		48.0		*40		
Symphysis								
Depth		52.5		*30.0		*34		
Thickness		20.0		15.0		*16		
At P ₄								
Depth		43.5	34.0	30.5		*28		
Thickness		15.5	15.0	13.0		*14		
At M ₃								
Depth		42.5		31.0	30.5			
Thickness		24.0		20.0	23.5			

*Estimated values.

GSP 4664, shows a number of equivocal morphological features and is not included in any of the three groups.

None of these specimens was found in direct association with cranial or dental material. Because only three different sized primate species are definitely represented by cranial and dental specimens, however, the largest postcranial specimen is provisionally assigned to *Gigantopithecus* cf. *bilaspurensis*, the intermediate sized group to *Sivapithecus indicus*, and the smaller specimens to *Ramapithecus punjabicus* (remembering that some of these specimens may represent juveniles of the middle size group). It is significant that the *Gigantopithecus* humeral fragment and many of the *Sivapithecus* specimens come from locality 311; dental remains from that locality include our only *Gigantopithecus* specimen and five *Sivapithecus indicus* (Table 2).

If the postcranial remains are correctly subdivided and if they are associated with the three size groups based on gnathic and dental remains, some interesting conclusions can be drawn.

First, all hominoid species in the Siwaliks apparently have cheek teeth with very thick occlusal enamel. Second, all are truly megadont, in that cheek teeth are very large relative to body size. In these two features, the Siwalik hominoids resemble Plio-Pleistocene hominids and differ from pongines and the Miocene hominoids now assigned to Dryopithecinae (*sensu strictu*, see below). Third, these species share a basically similar occlusal morphology.

The Siwalik hominoids subdivide into two groups, mainly on the basis of anterior tooth size. *S. indicus* has relatively large incisors and, like *S. sivalensis*, large dimorphic canines; *Ramapithecus* and *Gigantopithecus* species have relatively small incisors, (probably) non-projecting and (probably) moderately dimorphic canines.

Other hominoid material

New ideas and new hominoid fossil remains recovered during the past decade suggest that earlier, simpler schemes of hominoid evolution need to be modified. These changes can be summarised briefly as follows.

(1) Important dental differences between later hominids and living pongids lie less in arcade shape and incisor size as often stated in the past, but in relative tooth size and occlusal enamel thickness. Living apes have U-shaped dental arcades, living humans parabolic ones. Such arcade shapes are rarely found in other Neogene hominoids, the predominant form being some variant of a V-shape. Incisor size also seems to have been rather variable at all phases of hominoid evolution. More important, the apes, like almost all other non-human higher primates, have relatively small cheek teeth with thin enamel, perhaps an adaptation to predominantly browsing diets. Pliocene and earlier Pleistocene hominids in contrast have large cheek teeth with thick enamel (*Homo sapiens* has evolved small cheek teeth relatively recently). Apes have large tusk-like sexually dimorphic canines; hominids have small, somewhat incisiform canines exhibiting considerably less sexual dimorphism.

(2) New discoveries in east Africa, and new analyses of earlier finds in Africa and Europe, suggest that early apes were considerably more diverse than previously believed¹⁵. Classifying them in only one genus, *Dryopithecus*, obscures this diversity. Thus at least three genera (*Proconsul*, *Rangwapithecus* and *Limnopithecus*) should probably be recognised in east Africa during the early and middle Miocene, separated from *Dryopithecus*, at least two species of which are found in middle Miocene deposits in Europe. These species can all be placed conveniently in the Dryopithecinae (or Dryopithecidae). All have dentitions basically like those of the living African pongids, with thin enamel and, if postcranial remains are correctly allocated, relatively small cheek teeth. Canine-premolar complexes are like those of modern apes.

Postcranial material of this group has proved difficult to interpret in a framework heavily dependent on comparisons with modern primate groups¹⁶. The fossil species were probably arboreal, and seem to be qualitatively different from living apes, being more 'monkey-like' in certain ways; they are probably best

viewed as truly primitive relative to modern hominoids.

Where palaeoecological contexts can be inferred plausibly, dryopithecine species seem to have been associated with predominantly forest floras and faunas¹⁵. Adaptively this rather diverse group was probably more like living ceboids or cercopithecoids than the low-diversity modern hominoids.

(3) Besides dryopithecines, other kinds of hominoids are present in the Old World middle Miocene. New material from Hungary¹⁷, Greece¹⁸, Turkey¹⁹, Kenya²⁰ and China together with the specimens described here from Pakistan as well as earlier fossils from Europe and Africa show that thick-enamelled and (at least for those with postcranial remains) megadont hominoid species were widely distributed between 14 and 8 Myr ago, probably in predominantly non-forested habitats. During this time cercopithecoid monkeys seem to have been absent from Eurasia, and not particularly diverse in Africa²¹.

This thick-enamelled middle Miocene cluster of species can be divided into two groups. One, consisting of species variously described as *Sivapithecus*, *Bodvapiithecus*, *Ankarapithecus* and *Ouranopithecus* contains forms that are rather more ape-like, with large and sexually dimorphic canines. More than one generic name is probably necessary to reflect adequately the diversity of this group, which would be termed Sivapithecinae (or Sivapithecini). The other group would contain species described as *Gigantopithecus*, *Ramapithecus* and *Rudapithecus* (at least), forms with canines smaller and less dimorphic than those of pongines, dryopithecines or *Sivapithecus*-group species, although perhaps more so than australopithecines. Canine-premolar complexes resemble those of 'primitive' australopithecines²². This cluster would be termed Ramapithecinae (or Ramapithecini).

(4) Recent discoveries suggest very strongly that the story of the Plio-Pleistocene hominids was more complex than previously thought. Between about 3.75 and 1 Myr ago several hominid lineages seem to have coexisted, certainly in Africa and possibly in Asia too²³. These species have been classified in both *Australopithecus* and *Homo*, but are characterised by certain shared features: reduced canines exhibiting moderate size dimorphism, enlarged and thick-enamelled cheek teeth, relatively enlarged brains (compared with dryopithecines and pongines at least), and a postcranial skeleton showing numerous adaptations to habitual bipedalism. At least one of these species, by at least 2 and perhaps 2.5–3 Myr ago, made stone tools.

It has been increasingly realised that these so-called Plio-Pleistocene hominids are not to be regarded merely as 'diminutive humans' but as creatures, although recognisably hominid, qualitatively different from middle and late Pleistocene hominids. They are a diverse group of truly primitive species.

The oldest specimens with teeth similar to those of *Australopithecus* or early *Homo* species (and distinguishable from those of the *Sivapithecus* or *Ramapithecus* groups) come from Lukeino and Lothagam in Kenya and are between 5 and 7 Myr old^{24,25}.

The period after about 7 Myr ago is one during which australopithecines and early hominines diversified; *Sivapithecus*-group and *Ramapithecus*-group species are so far unknown. In both Africa and Eurasia cercopithecoid monkeys, especially the more open-country types, become abundant for the first time.

Synthesis

Current knowledge of hominoid evolution suggests a tentative taxonomic and phylogenetic scheme that reflects the important advances of the past decade. These are summarised here by D.P.

First, Neogene hominoids were, during most of their evolution, a relatively diverse superfamily. Second, extinct hominoids were not identical with, nor, in some cases, particularly similar to living hominoids, and to interpret extinct hominoids as though they were very 'modern' is potentially misleading. Third, it is very difficult to draw exact ancestor-descendant relationships given the complexity of the picture at any given time, the differences between descendants and available ancestral candidates, and the still substantial gaps in the fossil record. Rather, each radiation should be studied for its own sake in order to understand it as an adapted and successful group. Fourth, clearly the two taxonomic

categories into which living large hominoids are normally subdivided, Pongidae and Hominidae, cannot be imposed on earlier Neogene species without suppressing important information and obscuring evolutionary concepts.

A minimum of six clusters of species is needed to describe the diversity of Neogene large hominoids, and these can be classified as tribes or subfamilies.

(1) Ponginae: the living great apes. (2) Dryopithecinae: species from Africa and Europe, ranging in age from about 23 to 9 Myr, sharing dental features with pongines but differing in many postcranial characters; (3) Sivapithecinae or Sivapithecini: species from Eurasia and Africa, ranging in age from around 15 to 8 Myr, sharing dental features with both pongines and dryopithecines on the one hand and with australopithecines and early hominines on the other; (4) Ramapithecinae or Ramapithecini: Eurasian and African species resembling sivapithecines and australopithecines about equally in dental features; (5) Australopithecinae: species from Africa and possibly Asia ranging in age from at least 3.75 (and perhaps as much as 7) to 1 Myr; (6) Homininae: essentially similar to Australopithecinae in dental, cranial and postcranial morphology.

Figure 4 shows these groups distributed along temporal and qualitative morphological axes; the latter axis is shown as unidimensional although it is, of course, multidimensional. Three alternative phylogenies are indicated on the diagram in order of decreasing probability (in my opinion). Although definite relationships between the middle Miocene and Plio-Pleistocene hominoids are unclear because of gaps in the hominid record between 8 and 4 Myr and the pongid record after 9 Myr, I believe that the major groups as defined here represent at least some of the grades through which modern hominoids evolved. Phylogeny A is the most probable; I regard phylogenies B and C as less probable, although plausible.

I suggest the following classification of large Hominoidea, a compromise of 'vertical' and 'horizontal' philosophies²⁶. (1) Pongidae; (2) Dryopithecidae: including Dryopithecinae and older, more primitive hominoids; (3) Ramapithecidae: Sivapithecinae (or Sivapithecini) and Ramapithecinae (or Ramapithecini);

(4) Hominidae: Australopithecinae and Homininae.

The classification is flexible in that it is compatible with any of the three phylogenies suggested in Fig. 4. Whether or not the four groups are classified as families, subfamilies or tribes, they should be coordinate. As the fossil record improves I expect that certain (perhaps known) members of Ramapithecidae and Hominidae will prove more conclusively to be ancestors and descendants (that is phylogeny A in Fig. 4 becomes more probable), in which case some workers might wish to include those (or all) ramapithecids in Hominidae. Finally, two important points should be re-emphasised. A rigidly dichotomous classification of Neogene large hominoids is not only inappropriate but potentially misleading. And the living hominoids are rather aberrant forms when the total array of Neogene large hominoids is considered.

This work was supported by NSF, SFCP, the Government of Pakistan, the Geological Survey of Pakistan, Yale University and NERC. Special thanks go to E. L. Simons and B. Lipschutz.

Received 8 August; accepted 11 October 1977.

1. Pilbeam, D. *et al.* *Nature* **270**, 684-689 (1977).
2. Pilbeam, D. *Les Plus Anciens Hominidés* (eds Tobias, P. V. and Coppens, Y.) 39-59 (Centre National de la Recherche Scientifique, Paris, 1976).
3. Berggren, W. A. & Van Couvering, J. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **16**, no. 1/2 (1974).
4. Krynine, P. *Am. J. Sci.* **34**, 422-446 (1937).
5. Lewis, G. E. *Am. J. Sci.* **33**, 191-204 (1937).
6. Tattersall, I. *Nature* **221**, 451-452 (1969); **224**, 821-822 (1969).
7. Prasad, K. N. *Nature* **232**, 413-414 (1971).
8. Banerjee, D. *Rev. Palaeobotan. Palynol.* **6**, 171-176 (1968).
9. Nandi, B. *Himalayan Geol.* **5**, 411-424 (1975).
10. Johnson, G. D. *Geol. Rundschau* **66**, 192-216 (1977).
11. Schaller, G. *The Deer and the Tiger* (Chicago University Press, 1967).
12. Simons, E. L. *J. Hum. Evol.* **5**, 511-528 (1976).
13. Simons, E. L. *Proc. natn. Acad. Sci. U.S.A.* **51**, 528-535 (1964).
14. Pilgrim, G. E. *Rec. Geol. Surv. India* **45**, 1-74 (1915).
15. Andrews, P. & Van Couvering, J. *Approaches to Primate Paleobiology* (ed. Szalay, F.), 62-103 (Karger, Basel, 1975).
16. McHenry, H. & Corruccini, R. *Folia Primat.* **23**, 227-244 (1975).
17. Kretzoi, M. *Nature* **257**, 578-581 (1975).
18. de Bonis, L. & Melentis, J. C. *r. Lebd. Séanc. Acad. Sci.* **284**, 1393-1396 (1977).
19. Tekkaya, I. *Bull. Min. Res. Expl. Inst. Turkey* no. 83, 148-165 (1974).
20. Andrews, P. & Walker, A. in *Human Origins* (eds Isaac, G. & McCown, E.) 279-304 (Benjamin, New York, 1976).
21. Delson, E. *Approaches to Primate Paleobiology* (ed. Szalay, F.) 167-217 (Karger, Basel, 1975).
22. Johanson, D. & Taieb, M. *Nature* **260**, 293-297 (1976).
23. Leakey, R. & Walker, A. *Nature* **261**, 572-574 (1976).
24. Behrensmeyer, A. *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, F., Isaac, G. & Leakey, R.) 163-170 (Chicago University Press, 1976).
25. Pickford, M. *Nature* **256**, 279-284 (1975).
26. Simpson, G. G. *Principles of Animal Taxonomy* (Columbia University Press, New York, 1961).

A mutation that impairs the ability of lipoprotein receptors to localise in coated pits on the cell surface of human fibroblasts

Richard G. W. Anderson, Joseph L. Goldstein & Michael S. Brown

Departments of Cell Biology and Internal Medicine, University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Low density lipoprotein is taken up by cultured human fibroblasts through an endocytic process that requires the binding of the lipoprotein to specific receptors located in coated pits on the cell surface. The coated pits are discrete segments of the plasma membrane that can undergo rapid invagination to form coated endocytic vesicles. In one form of the human genetic disorder familial hypercholesterolaemia, the responsible mutation produces altered lipoprotein receptors that lack the ability to become incorporated into coated pits. Instead, these mutant receptors are scattered at random over the entire plasma membrane. Because of their mislocation on the cell surface, the mutant lipoprotein receptors are unable to carry their bound lipoprotein into the cell. The occurrence of this 'receptor mislocation mutation' provides strong evidence for the role of the coated pit in the receptor-mediated uptake of lipoproteins. The data also have implications for the structure and assembly of plasma membranes in mammalian cells.

COATED pits on the cell surface constitute specialised membrane regions that mediate the uptake of macromolecules through adsorptive endocytosis¹⁻⁵. In cultured human fibroblasts, these coated pits appear in thin-section electron micrographs as short (0.5 μ m) segments of the plasma membrane where the membrane is indented, thickened, and coated on its cytoplasmic surface by a filamentous material that frequently assumes a spike-like configuration^{4,6,7}. Using the freeze-fracture/deep-etching technique, the coated pits have also been visualised in two dimensions on the fibroblast surface where they appear as shallow, crater-like depressions that occupy about 2% of the surface area. In freeze-fracture preparations, the membrane of these coated pits can be distinguished from the membrane in noncoated regions by the presence of a higher concentration of intramembranous particles^{2,5}.

Coated pits as site of lipoprotein receptors

In human fibroblasts, coated pits of the plasma membrane have been shown by both conventional transmission electron microscopy^{4,6,7} and freeze-etching techniques^{2,5} to contain specific receptors for low density lipoprotein (LDL), the predominant

transport protein for cholesterol in human plasma. Through the use of LDL covalently coupled to ferritin, we recently demonstrated that 50–80% of LDL receptors are concentrated within the 2% of cell surface that constitutes the coated pits^{4,6,7}. The LDL-ferritin bound to its receptor in coated pits is internalised within several minutes when the coated pits invaginate to form coated endocytic vesicles⁴. The coated vesicles containing LDL migrate through the cytoplasm until they fuse with lysosomes⁴. The lysosomal acid hydrolytic enzymes then cleave the cholesteryl esters and release the free cholesterol of the lipoprotein. The liberated sterol becomes available for metabolic utilisation by the cell (for review, see refs 8, 9).

On the basis of previous studies, we postulated that the rapid internalisation of receptor-bound LDL is strictly dependent on the localisation of LDL receptors in coated pits⁴. We also suggested that these coated pits may contain other cell surface receptors and that the invagination of these pits into the cell every few minutes would provide a mechanism by which a variety of receptor-bound molecules could be transported rapidly into the cell and delivered to lysosomes⁴. A corollary of this hypothesis is that if a receptor were not contained within such a coated pit region, then it would not be able to transport its bound ligand to cellular lysosomes with high efficiency.

Role of R^{b+i} allele in internalisation of LDL

Recently, a human mutation that allows testing of the above hypothesis has been discovered. This mutation was observed in fibroblasts cultured from a 14-yr-old (J.D.) who has the clinical phenotype of homozygous familial hypercholesterolaemia (FH), a hereditary disorder characterised by a marked increase in the plasma level of LDL¹⁰. Unlike 30 other subjects with the homozygous form of FH whose fibroblasts exhibit a primary defect in the binding of LDL to the receptor¹¹, J.D.'s cells bind normal amounts of ¹²⁵I-labelled LDL at the receptor site, yet the receptor-bound lipoprotein fails to be internalised and consequently it is not degraded within cellular lysosomes¹⁰. This uptake defect is specific for LDL since the J.D. cells show normal bulk-fluid endocytosis of ¹⁴C-sucrose and ¹²⁵I-labelled gamma globulin¹⁰ as well as normal receptor-mediated binding, internalisation, and lysosomal degradation of ¹²⁵I-labelled human epidermal growth factor, a molecule that is metabolised by human fibroblasts in a manner similar to LDL (ref. 12 and our unpublished observations).

Biochemical studies of the fibroblasts of J.D.'s family members have disclosed that J.D. possesses two different mutant alleles at the LDL receptor locus¹³. From his mother, he has inherited the R^{b^*} allele that specifies a receptor molecule that is unable to bind ¹²⁵I-LDL normally and is thus biochemically silent. From his father, he has inherited the R^{b+i} allele that specifies a receptor that can bind ¹²⁵I-LDL normally but cannot facilitate the internalisation of the receptor-bound lipoprotein. Because these two defects fail to complement each other in J.D. (genotype, R^{b^*}/R^{b+i}), we reasoned that they represent allelic mutations that disrupt different sites on the LDL receptor protein. The R^{b^*} mutation involves the site necessary to bind LDL, and the R^{b+i} mutation involves a site necessary to couple the binding and internalisation processes¹³.

R^{b+i} allele is a 'receptor mislocation mutation'

The discovery of the R^{b+i} allele raises intriguing questions as to the relation between the mutant receptors specified by that allele and the coated pit of the plasma membrane. Accordingly, we have used LDL labelled with ferritin and ¹²⁵I to examine the localisation of LDL receptors in the J.D. cells at the electron microscopic and light microscopic levels, respectively. The results indicate that the R^{b+i} mutation in the J.D. cells affects the LDL receptor in such a way that it cannot be incorporated into coated pits. The resultant mislocation of LDL receptors on the cell surface precludes the endocytosis of the receptor-bound lipoprotein.

Figure 1a shows a deeply invaginated coated pit of a normal fibroblast as it appears in thin sections of cells that have been incubated with ferritin-labelled LDL for 2 h at 4 °C, then fixed, and embedded while still in monolayer culture. Typically, most of the LDL-ferritin particles (open arrow) are clustered within the coated pit. In this experiment approximately 85% of the identifiable coated pits contained LDL-ferritin particles. In the J.D. fibroblasts (Fig. 1b), the coated pits were indistinguishable from the coated pits observed in normal cells (Fig. 1a and refs 4, 6, 7). In particular, their size, configuration, and distribution over both the top and bottom surfaces of the cell appeared the same in the J.D. and normal fibroblasts. In two experiments in which at least 2 mm of cell surface were examined per experiment, the number of coated pits per mm in the normal and J.D. cells incubated at 4 °C averaged 27 (25 and 29) and 19 (22 and 16), respectively. In two experiments in which the cells were incubated at 37 °C, the number of coated pits per millimetre of cell surface in the normal and J.D. cells averaged 13 (11 and 14) and 10 (10 and 10), respectively. The lower number of identifiable coated pits in normal cells at 37 °C as compared to 4 °C has been noted in previous studies and presumably is due to an altered morphology at the two temperatures⁴.

Despite a normal appearance and nearly normal number of coated pits in the J.D. cells, virtually no LDL-ferritin was bound in these specialised membrane regions (Fig. 1b). Rather, as shown in Fig. 1c and d, in the J.D. cells LDL-ferritin particles (open

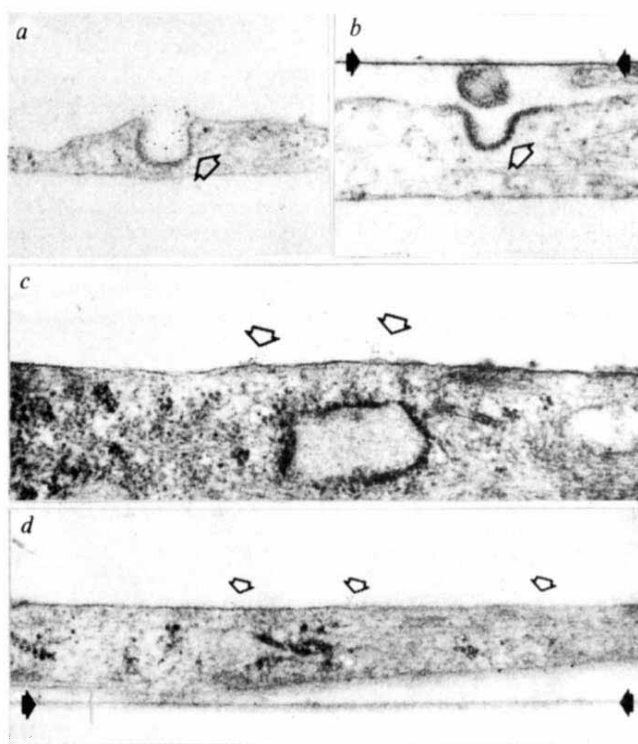


Fig. 1 Electron micrographs that show the localisation of LDL-ferritin on the cell surface of normal (a) and J.D.'s (b–d) fibroblasts. Monolayers of fibroblasts were prepared in 60 × 15 mm Petri dishes as previously described⁴ and used for experiments in the late logarithmic phase of growth after a 48-h incubation in the presence of 5% (v/v) human lipoprotein-deficient serum²³. On the day of the experiment (day 7 of cell growth), monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% human lipoprotein-deficient serum and LDL-ferritin at a concentration corresponding to 58 $\mu\text{g ml}^{-1}$ of LDL-protein. After incubation at 4 °C for 2 h, each monolayer was washed five times at 4 °C with ice-cold phosphate-buffered saline, fixed *in situ* with 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3), embedded *in situ*, sectioned, and stained for electron microscopy^{4,6}. The LDL-ferritin complex was prepared as previously described^{4,6}. By negative staining electron microscopy, 2 to 3 ferritin particles were coupled to each LDL particle. The open arrows denote LDL-ferritin particles. The solid arrows denote the layer of serum proteins that coats the surface of the culture dish. 58,000 ×.

Table 1 Quantitative analysis of binding of LDL-ferritin to coated pits of plasma membrane of normal and mutant fibroblasts

Cell strain	Genotype	Ferritin cores bound (no. per mm of cell surface)			% of bound ferritin cores associated with coated pits	¹²⁵ I-LDL bound (ng per mg protein)	
		In coated pits (a)	In non-coated regions (b)	Total (a + b)		Total	High affinity
Normal	+/+	186	195	381	49%	130	125
Normal	+/+	186	165	351	53%	121	116
J.D.	R ^b /R ^{b+,i}	10	342	352	3%	99	93
Father of J.D.	+ / R ^{b+,i}	112	444	556	20%	194	188

Cell monolayers were prepared as described in the legend to Fig. 1. On the day of the experiment (day 7 of cell growth), the fibroblast monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% lipoprotein-deficient serum and either LDL-ferritin (58 µg ml⁻¹ of LDL-protein) or ¹²⁵I-LDL (5 µg ml⁻¹ of LDL-protein, 212 c.p.m. per ng protein). The ¹²⁵I-LDL was added in the absence and presence of 300 µg protein per ml of unlabelled LDL. For the dishes that received LDL-ferritin, the monolayers were washed, fixed, embedded, and sectioned for electron microscopy as described previously^{4,6}. Quantitation of the LDL-ferritin binding was performed on unstained sections as previously described⁶ except that the sections were mounted directly from the diamond knife boat on to 100/400 mesh grids. Each value represents the average of duplicate monolayers. A total of about 2 mm of cell surface membrane was counted for each monolayer. For the dishes that received ¹²⁵I-LDL, the monolayers were washed six times at 4 °C with an albumin-containing buffer¹⁴, and the amounts of total and high affinity ¹²⁵I-LDL bound to the cell surface were determined as previously described¹⁴. The values for high affinity ¹²⁵I-LDL binding were determined by subtracting values for nonspecific binding from the values for total binding. The values for nonspecific binding represents the amount of ¹²⁵I-LDL bound to the monolayers in the presence of the 60-fold excess of unlabelled LDL. All values represent the average of data obtained from duplicate monolayers.

arrows) were bound in a random fashion at intervals along the plasma membrane remote from the coated pits.

Quantitative analysis of LDL-ferritin binding

To confirm these qualitative findings by quantitative methods, fibroblasts from two normal subjects (+/+), from J.D. (R^b/R^{b+,i}), and from J.D.'s heterozygous father (+/R^{b+,i}) were incubated with LDL-ferritin at 4 °C, washed extensively to remove nonspecifically bound material, and fixed in monolayer culture. Sections of the cells were prepared and examined at random by an observer who was unaware of the source of the specimens and the experimental design. Linear segments of plasma membrane totalling 4 mm in length were examined and the number of LDL-ferritin particles localised within coated pits or found on non-coated regions was recorded. As a part of the same experiment, one set of dishes from each genotype was incubated with ¹²⁵I-LDL and the amounts of total and high affinity ¹²⁵I-LDL binding to the LDL receptor were measured biochemically at 4 °C as previously described¹⁴. Table 1 shows that in the two normal cell strains (+/+), approximately 50% of the LDL-ferritin was bound over recognisable coated pits. In the J.D. cells (R^b/R^{b+,i}), the total amount of LDL-ferritin binding (as well as that of ¹²⁵I-LDL) was only slightly lower than that in the normal cells, but only 3% of the LDL-ferritin particles were found in coated pits. In the cells from J.D.'s father (+/R^{b+,i}), the total amount of LDL-ferritin binding (as well as that of ¹²⁵I-LDL) was greater than that in the normal cells (Table 1). But, the percentage of LDL-ferritin bound in coated pits was only half as much as in normal cells (20% compared with 50%). This latter ultrastructural finding is consistent with previous biochemical data demonstrating that the cells of J.D.'s father contain two populations of LDL receptors: one population (the product of the normal allele) that internalises LDL normally¹³ and is presumably incorporated normally into coated pits and a second population (the product of the R^{b+,i} allele) that fails to internalise receptor-bound LDL¹³ and, in the current studies, seems to be excluded from the coated pits.

The quantitative and qualitative electron microscopic observations indicate that the defective internalisation of LDL in the J.D. cells is due to an inability of the LDL receptors specified by the R^{b+,i} allele to localise in coated pits. This conclusion was supported by additional experiments in which the disappearance of LDL-ferritin from the cell surface was quantified. In one of these experiments, normal cells and J.D. cells were incubated with LDL-ferritin at 4 °C for 2 h, washed, and then warmed to 37 °C for varying intervals before fixation. At each interval, the number of LDL-ferritin particles remaining on the cell surface was counted as previously described^{4,6}. In the normal cells the number of surface-bound LDL-ferritin particles declined by 75% after the cells had been warmed for only 2 min. As previously

reported⁴, this disappearance of LDL-ferritin from the surface of normal fibroblasts is attributable to the internalisation of the lipoprotein in coated endocytic vesicles. In contrast to the results in the normal cells, the amount of LDL-ferritin bound to the

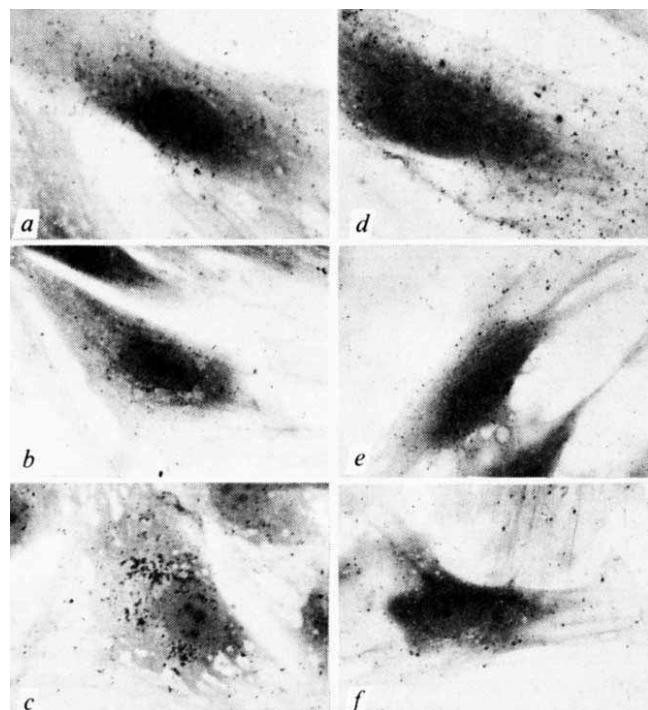


Fig. 2 Autoradiograms of normal (a-c) and J.D.'s (d-f) fibroblasts exposed to ¹²⁵I-LDL for 2 h at 4 °C and then warmed to 37 °C for various times. Monolayers of fibroblasts were prepared in 60 × 15 mm Petri dishes and incubated for 48 h in 5% lipoprotein-deficient serum as described in Fig. 1 except that the cells were grown on glass coverslips (22 × 22 mm) as previously described⁴. On the day of the experiment (day 7 of cell growth), monolayers were cooled to 4 °C for 30 min. The medium was removed and replaced with 2 ml of ice-cold growth medium containing 5% human lipoprotein-deficient serum and 10 µg protein ml⁻¹ of ¹²⁵I-LDL (276 c.p.m. per ng protein)²⁴. The monolayers were incubated at 4 °C for 2 h, after which each dish was washed six times at 4 °C with an albumin-containing buffer as previously described¹⁴. Some sets of coverslips were fixed immediately (a and d), while others received 2 ml of growth medium containing 5% human lipoprotein-deficient serum and were warmed to 37 °C in the Petri dish for either 10 min (b and e) or 60 min (c and f) before fixation. The fixative was 6% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.3)⁴. The coverslips were prepared for autoradiography as previously described⁴. The exposure time was 2 weeks at 4 °C. Autoradiograms were examined with an American Optical Microstar 10 and photographed with a Polaroid camera. 1,000 ×.

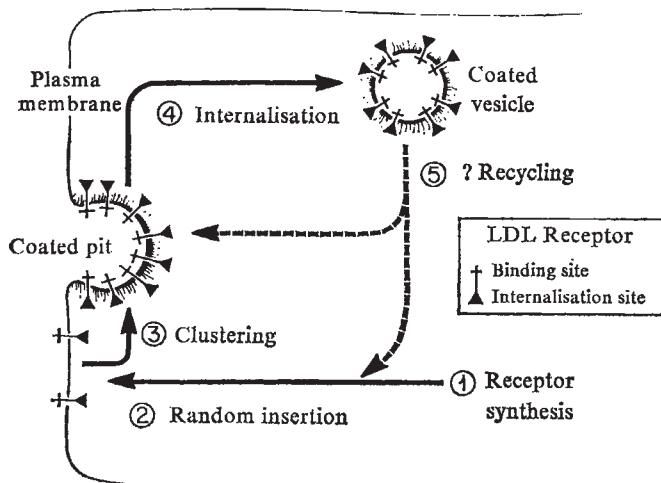


Fig. 3 Schematic illustration of the proposed pathway by which LDL receptors become localised to coated pits on the plasma membrane of human fibroblasts. The sequential steps in this process are as follows: (1) synthesis of LDL receptors on polyribosomes, (2) insertion of LDL receptors at random sites along non-coated segments of plasma membrane, (3) clustering together of LDL receptors in coated pits, (4) internalisation of LDL receptors as coated pits invaginate to form coated endocytic vesicles, and (5) recycling of internalised LDL receptors back to the plasma membrane.

surface of the J.D. cells declined by less than 5% even after 10 min of incubation at 37 °C.

Localisation of LDL receptors by autoradiography

The failure of internalisation of LDL in the J.D. cells could also be demonstrated by light microscopic autoradiography using ^{125}I -LDL. Figure 2a shows a normal fibroblast incubated for 2 h at 4 °C with ^{125}I -LDL and then prepared for autoradiography. The ^{125}I -LDL is distributed over the entire cell surface. In the J.D. cells the ^{125}I -LDL was bound similarly (Fig. 2d). When the normal cells were warmed to 37 °C for 10 min (Fig. 2b) or 60 min (Fig. 2c), there was a progressive increase in the radioactivity over the perinuclear area. Previous studies have shown that this migration of silver grains reflects the internalisation of the ^{125}I -LDL and its transport to cellular lysosomes⁴. In contrast, when the J.D. cells were warmed for 10 min (Fig. 2e) or 60 min (Fig. 2f), there was no increase of the ^{125}I -LDL in the perinuclear region. There was a slight reduction in the number of silver grains over the periphery of the J.D. cells with time at 37 °C (compare Fig. 2d and f). Previous biochemical measurements have shown that this is due to a slow dissociation of intact ^{125}I -LDL from the receptor sites at 37 °C¹⁰.

Implications for the mechanism of interaction of cell surface receptors with coated pits

Our results support the concept that the coated pit is a specialised region of the plasma membrane whose function is to undergo internalisation at a rapid rate and to carry receptor-bound molecules into the cell. The LDL receptors specified by the $R^{b+,i}$ allele lack the ability to become localised within these coated pits. As a result, when LDL binds to these mutationally altered receptors, it is not carried into the cell and remains bound to the cell surface over non-coated regions of the plasma membrane. The mislocation of the mutant receptors specified by the $R^{b+,i}$ allele can be demonstrated conveniently in the cells of J.D. ($R^b/R^{b+,i}$) because the partner allele at the LDL receptor locus in J.D. is also mutant (R^b), specifying a receptor that does not bind LDL¹³.

The manifestations of the $R^{b+,i}$ mutation suggest that the incorporation of LDL receptors into coated pits may be analogous to the process by which membrane proteins of certain lipid-containing viruses are inserted into patches of host plasma membrane before the budding of the virus from the host cyto-

plasm¹⁵. In one of the best studied of these viral systems, that of vesicular stomatitis virus, the viral surface glycoprotein, G, is a transmembrane protein that is synthesised on host membrane-bound polyribosomes, glycosylated, and is then inserted at random into the host plasma membrane¹⁵⁻¹⁷. These G proteins are then clustered together as a patch in association with another viral protein, M, which is synthesised in the cytoplasm and coats the cytoplasmic side of the plasma membrane. Under the direction of the M protein, the nucleocapsid of the virus attaches to the patch of viral proteins and the plasma membrane then buds off to form a complete viral particle. In the process of coalescence of the G and M proteins, the host cell's own plasma membrane proteins are excluded from the viral patch, so that the mature viral membrane contains mainly virus-coded proteins¹⁵⁻¹⁷.

Figure 3 depicts a proposed working model for the incorporation of LDL receptors into coated pits that is based on the above model for viral membrane formation and on current concepts of membrane structure^{18,19}. The LDL receptor is envisaged as a transmembrane protein that is synthesised on membrane-bound polyribosomes and inserted at random sites in the plasma membrane, probably after glycosylation in the Golgi apparatus. The external portion of the LDL receptor contains the binding site for LDL. The cytoplasmic portion contains a specific amino acid sequence that is necessary for recognition of the receptor as a component of coated pits. After their insertion into the plasma membrane, the receptor molecules that contain the proper recognition sequence are gathered together and incorporated into coated pits under the influence of unknown peripheral membrane proteins that interact at the cytoplasmic surface of the membrane. It is possible that clathrin, the protein that has been identified as the major component of the cytoplasmic coat of coated vesicles^{20,21}, functions in this capacity.

Several aspects of the model in Fig. 3 are worthy of emphasis. First, earlier data indicate that the clustering of LDL receptors into coated pits is not dependent on the binding of LDL^{4,6}. Thus, when fibroblasts are fixed with formaldehyde before incubation with LDL-ferritin, the majority of receptor sites are still found within coated pits⁶. It therefore seems that the sequence of receptor insertion into the plasma membrane, clustering into coated pits, and internalisation proceeds continuously whether or not LDL is present. Second, kinetic data strongly suggest recycling of internalised LDL receptors back to the cell surface. Thus, although nearly all of the identifiable LDL receptors on the cell surface enter cells within 10 min, they are immediately replaced with an equal number of functionally active receptors^{9,10,14}. This internalisation and replacement continues at an uninterrupted rate for at least 6 h even when protein synthesis is inhibited totally with cycloheximide (unpublished).

The model in Fig. 3 helps to explain the defect in the mutant receptor produced by the $R^{b+,i}$ allele. We postulate that this receptor is defective in its cytoplasmic portion that contains the recognition site for incorporation into coated pits. As a result, these mutant receptors are not recognised by the cytoplasmic proteins that form coated pits, they remain scattered at random along the cell surface, and hence they fail to internalise LDL.

As stated above, it seems likely that coated pits contain receptors for other macromolecules in addition to LDL. For example, the kinetics of binding, internalisation, and lysosomal degradation of epidermal growth factor¹² and transcobalamin II²² in human fibroblasts are identical with those of LDL. In each case, essentially all of the receptor-bound ligand enters the cell within 10 min. On the basis of the model proposed in Fig. 3, we hypothesise that the incorporation of each of these receptors into coated pits involves a mechanism similar to that proposed for the LDL receptor.

This research was supported by a grant from the US NIH (HL-20948). We thank Debra Lieberman and Gloria Y. Brunschede for technical assistance. Marian Eastman and Jean Helgeson provided help with the tissue culture.

Received 8 August; accepted 24 October 1977.

1. Roth, T. F. & Porter, K. R. *J. Cell Biol.* **20**, 313-332 (1964).
2. Fawcett, D. W. *J. Histochem. Cytochem.* **13**, 75-91 (1965).

3. Friend, D. S. & Farquhar, M. G. *J. Cell Biol.* **35**, 357–376 (1967).
4. Anderson, R. G. W., Brown, M. S. & Goldstein, J. L. *Cell* **10**, 351–364 (1977).
5. Roth, T. F., Cutting, J. A. & Atlas, S. B. *J. supramolec. Struct.* **4**, 527–548 (1976).
6. Anderson, R. G. W., Goldstein, J. L. & Brown, M. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2434–2438 (1976).
7. Goldstein, J. L., Brown, M. S. & Anderson, R. G. W. in *International Cell Biology 1976–1977* (eds Brinkley, B. R. & Porter, K. R.) 639–648 (Rockefeller University Press, New York, 1977).
8. Brown, M. S. & Goldstein, J. L. *Science* **191**, 150–154 (1976).
9. Goldstein, J. L. & Brown, M. S. *Curr. Top. cell. Reg.* **11**, 147–181 (1976).
10. Brown, M. S. & Goldstein, J. L. *Cell* **9**, 663–674 (1976).
11. Brown, M. S. & Goldstein, J. L. *N. Engl. J. Med.* **294**, 1386–1390 (1976).
12. Carpenter, G. & Cohen, S. *J. Cell Biol.* **71**, 159–171 (1976).
13. Goldstein, J. L., Brown, M. S. & Stone, N. J. *Cell* **13**, 629–641 (1977).
14. Goldstein, J. L., Basu, S. K., Brunschede, G. Y. & Brown, M. S. *Cell* **7**, 85–95 (1976).
15. Lenard, J. & Compans, R. W. *Biochim. biophys. Acta* **344**, 51–94 (1974).
16. Knipe, D. M., Lodish, H. F. & Baltimore, D. *J. Virol.* **21**, 1121–1127; 1140–1148 (1977).
17. Knipe, D. M., Baltimore, D. & Lodish, H. F. *J. Virol.* **21**, 1128–1139; 1149–1158 (1977).
18. Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43–49 (1975).
19. Singer, S. J. & Nicolson, G. L. *Science* **175**, 720–731 (1972).
20. Pearse, B. M. F. *J. molec. Biol.* **97**, 93–98 (1975).
21. Pearse, B. M. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1255–1259 (1976).
22. Youngdahl-Turner, P., Allen, R. H. & Rosenberg, L. E. *Chm. Rev.* **25**, 472A (1977).
23. Brown, M. S., Dana, S. E. & Goldstein, J. L. *J. biol. Chem.* **249**, 789–796 (1974).
24. Brown, M. S. & Goldstein, J. L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 788–792 (1974).
25. Onci *et al. Expl Cell Res.* (In the press).

letters to nature

Evolution of dynamical systems with time-varying gravity

VARIOUS attempts have been made to place limits on the time-variability of gravity through observations of astrophysical objects. We demonstrate that contrary to previous studies^{1,2}, no useful limits can be obtained from observations of N -body systems such as galaxies and star-clusters.

Van Flandern³ has analysed over 21 years of lunar occultation data while Muller⁴ has studied ancient astronomical observations of eclipses from 1374 BC, to AD 1715. The results of these two independent experiments can be combined for cosmologies which conserve angular momentum to imply⁴

$$G/G = -2.6 \pm 1.5 \times 10^{-11} \text{ yr}^{-1} \quad (1)$$

where G is Newton's gravitational 'constant'. In addition, there are several recent field theories involving a time dependent G which are compatible with both general relativity and all Solar System tests of relativity^{5,6}.

In order to test these theories and explore the consequences of the above mentioned measurements, astrophysical effects of a time-dependent G must be examined in old astronomical objects that are dominated by gravity. For example, a recent analysis⁷ of the spindown of pulsars with varying G placed a limit on the possible weakening of gravity which is just compatible with equation (1).

In another effort to test varying G , a numerical study of the dynamics of clusters of galaxies and globular clusters was made which found¹ that $-G/G < 4 \times 10^{-11} \text{ yr}^{-1}$. In this letter we study the evolution of dynamical systems with time varying gravity and show that these calculations as well as similar studies² are incorrect. We also demonstrate rigorously that, unlike the case of pulsar spindown, the dynamics of clusters of galaxies and globular clusters cannot place any useful limits on G/G .

For convenience we can, without loss of generality, keep all masses constant, let only G depend on time, and measure time on atomic clocks. Then consider a system of point masses, m_i , with trajectories $\mathbf{R}_i(t)$. The (time-dependent) gravitational constant is $G(t) = G_0 a^{-1}(t)$; $G_0 \equiv G(0)$ with $t=0$ denoting the present epoch. The equation of motion of the i th particle is, in the limit of Newtonian mechanics,

$$\frac{d^2 \mathbf{R}_i}{dt^2} = \sum_{j \neq i} G(t) m_j \frac{(\mathbf{R}_j - \mathbf{R}_i)}{|\mathbf{R}_j - \mathbf{R}_i|^3} \quad (2)$$

We define a new time coordinate τ by

$$\tau(t) = \int_0^t a^{-2}(t') dt' \quad (3)$$

We describe the trajectories by $\mathbf{r}_i$, defined by $\mathbf{R}_i(t) = \mathbf{R}_c(t) + a(t)\mathbf{r}_i(t)$, where $\mathbf{R}_c$ is the centre of mass of the system. The equation of motion now becomes

$$\frac{d^2 \mathbf{r}_i}{d\tau^2} = \sum_{j \neq i} G_0 m_j \frac{(\mathbf{r}_j - \mathbf{r}_i)}{|\mathbf{r}_j - \mathbf{r}_i|^3} - \mathbf{r}_i a^3 \frac{d^2 a}{d\tau^2} \quad (4)$$

The last term in equation (4) is zero if $a(t)$ takes the usual form $a(t) = (t+T)/T$, where T is the age of the universe in atomic time as predicted by a given cosmology. When our results are specialised to the two-body problem using this form of $a(t)$, we recover Vinti's⁸ solution. For an N -body system with a more general form of $a(t)$ we define $t_G \equiv G/(dG/dt)$ ($t_G \approx T$ for cosmologies of interest) and define t_D as the dynamical time of the system. We then note that the relative importance of the last term in equation (4) is roughly

$$\frac{d^2 \mathbf{r}_i/d\tau^2}{r_i a^3 (d^2 a/d\tau^2)} \sim \frac{r/\tau_D^2}{ra^4/t_G^2} \sim t_G^2/t_D^2 \quad (5)$$

Thus, for any system in which $t_D \ll t_G \approx T$ (the condition required for virial equilibrium) the system evolves precisely as it would if G were constant, except that its size and time scales are proportional to $a(t)$ and $a^2(t)$, respectively. Thus, the 'qualitative' dynamical evolution of such equilibrium objects as galaxies, clusters of galaxies, and globular clusters is not affected by a time varying gravity in the Newtonian limit.

The homologous evolution of equilibrium gravitational systems described above is observable, in principle, because expansion (or contraction) would increase the r.m.s. velocity of the system by about r/t_G over the virial equilibrium value. The residual velocities would characteristically be proportional to distance from the centre of the system. But only when $t_D \ll t_G \approx T$ may we invoke the virial theorem. In such cases the residual velocities are much smaller than the r.m.s. velocities. Therefore, the virial discrepancy caused by a time-variable gravity would probably be unobservable, even if a system's mass could be measured independent of the virial theorem. Lewis² recognised that a time-variable gravity creates a virial discrepancy. But he applied this idea to loose associations of galaxies in which t_D is not small compared to t_G and for which the virial theorem may not be appropriate.

Our results contradict the work of Dearborn and Schramm¹. Their numerical results showed that individual members of an N -body system could escape as G decreased. Their scheme, however, ignored the evolution of the system as a whole, while following the orbits of single test particles. Thus, they were unable to recognise the homologous development of the system.

The present results do not apply to the latest version of the Dirac cosmologies⁵ since then equation (2) has an additional cosmological term which may produce qualitative changes in the system over cosmological time scales.

The current properties of collisionless equilibrium systems (such as galaxies) cannot be used to place limits on G because, as

we have shown, they undergo no qualitative evolution. Observations of systems where two-body relaxation is important, such as globular clusters, can in principle provide such limits because they do undergo qualitative changes with time. But neither observations of globular clusters nor the theory of dynamical cluster evolution are currently precise enough to produce limits comparable to those provided by experiments. We should emphasise that local laboratory and Solar System tests of $\dot{G}$ are quantitative in nature and not subject to the limitations inherent in qualitative dynamical tests.

We thank Professor S. Shapiro for helpful discussions. This work was supported in part by the Colgate Research Council and in part by NSF (grant AST-75-21153).

ALAN MARCHANT*

Department of Astronomy,
Cornell University,
Ithaca, New York 14853

VICTOR MANSFIELD

Department of Physics and Astronomy,
Colgate University,
Hamilton, New York 13346

Received 3 August; accepted 19 October 1977.

1. Dearborn, D. S. & Schramm, D. N. *Nature* **247**, 441–443 (1974).
2. Lewis, B. M. *Nature* **261**, 302–304 (1976).
3. Van Flandern, T. C. *Mon. Not. R. astr. Soc.* **170**, 333–342 (1975).
4. Muller, P. M. *Jet Propulsion Lab. Res. Report* (15 Sept. 1976).
5. Canuto, V., Adams, P. J., Hsieh, S.-H. & Tsiang, E. *Phys. Rev. D* (in the press).
6. Bekenstein, J. D. *Phys. Rev. D* **15**, 1458–1468 (1977).
7. Mansfield, V. N. *Nature* **261**, 560–562 (1976).
8. Vinti, J. P. *Mon. Not. R. astr. Soc.* **169**, 417–427 (1974).

Are supernovae sources of presolar grains?

ISOTOPIC measurements¹ in chondritic meteorites have revealed that live ²⁶Al was present in the early solar system. ²⁶Al and other reported isotopic peculiarities^{2–4} in the meteorite Allende could be attributable to explosive nucleosynthesis, sparking suggestions^{1–8} that a nearby supernova may have contaminated the early solar nebula. One explanation for isotopic anomalies in early nebular condensates is the injection of supernova grains. An advantage of this mechanism is that it could avoid excessive dilution of the anomalies by the bulk of the nebula. We examine here the early expansion of supernovae to determine the likelihood of grain nucleation and growth.

Models^{9–12} of type II supernovae (SN II), consisting chiefly of an extended low density superegiant envelope, have obtained good quantitative agreement with observationally determined¹³ light curve time scales, absolute magnitudes and expansion velocities. The light curve is generated by the passage of a strong shock wave through the envelope. The shock passage through the underlying mantle region may cause the production of anomalous isotopic compositions during explosive processing¹⁴ of these zones, so the evolution of the (hydrogen depleted) mantle is of major interest.

These models^{9–12} suggest the observed decline in luminosity at about 50–70 d results from recombination in and growing transparency of the expanding envelope. The mantle material may then be exposed, and several cases of significant light after this decline have been observed¹⁵. The inferred photospheric radii and effective temperatures fall in the range $R_{ph} \sim 1\text{--}3 \times 10^{14}$ cm, $T_e \sim 3\text{--}5 \times 10^3$ K, in agreement with calculations of light curves which include material inside the envelope (ref. 11 and S.F. in preparation). Such models indicate mantle densities of 3×10^{-15} to 10^{-13} g cm⁻³ by the time cooling to the condensation temperatures ($T_c \sim 1,000$ K) has occurred. Numerical nucleation calculations (J.L. and S.F., in preparation) which include mass conservation, expansion and cooling effects, and sputtering imply that grain formation occurs when $\phi > \phi_0 \equiv n f_c \alpha = 10^6$ cm⁻³. Here α is an effective sticking probability, f_c the number fraction of condensibles, and n the material number density. One condensing species has been assumed in these calculations, and no attempt to include subsequent coagulation, or chemical or temperature effects on α has been made. These calculations suggest that

Table 1 Model characteristics and predicted broadband fluxes at Earth

t (yr)	$(^\circ\text{K})$	T_e (K)	$R/10^{15}\text{cm}$	τ	$d^2 F_1^*(jz)$	$d^2 F_{10}^\dagger(jz)$
2.5	1000	1000	1	23	3(–8)	3.5(–5)
3.2	750	750	1.35	24	1(–10)	3.6(–5)
4.5	470	500	2	26	1(–14)	2.3(–5)
8.5	220	250	4	5	—	3.0(–6)
10.5	195	200	5	2	—	2.6(–6)
20.5	100	100	10	<.2	—	5.1(–9)

*Interval 0.9–1.2 μm , d in kpc.

†Interval 9–12 μm .

virtually all the mantle (which has $n f_c \sim 2\text{--}10 \times 10^6$ cm⁻³) may form grains a year or so after explosion.

The resulting size distributions are relatively flat and centred about sizes $a \sim 5 \times 10^{-8} \phi / \phi_0$ cm. Further growth through condensation is halted by monomer depletion, rather than by declining density as previously suggested¹⁶, an assumption which led to $a \sim 1\text{--}2 \times 10^{-5}$ cm. If post-nucleation growth by coagulation is important¹⁷, sizes of order $a \sim 1\text{--}3 \times 10^{-6}$ cm are predicted if the physical parameters of ref. 16 are assumed. But it is possible that C/O < 1 in the bulk of this ejecta¹⁸, so graphite will not condense and silicate grains dominate. Using our estimate for ϕ , the coagulation mechanism leads to $a \lesssim 10^{-6}$ cm. Thus SN II seem likely sites for the growth of small grains, perhaps containing some isotopically anomalous compositions. Incidentally, these grains are almost certainly too small to trap any fission products of the superheavy nuclei which have been suggested as responsible for the Xe anomalies in Allende^{19,20}.

The case for type I supernovae is not so favourable. These events exhibit light curves with a long-term exponential tail (up to 600 d, ref. 14) and seem to require a source of continuous heat or radiation input. No self-consistent mechanism for explaining these features in the absence of a strong continuum¹³ has yet been proposed. One suggestion^{21,22} is that ~ 0.1 M of ⁵⁶Ni could supply this energy by radioactivity. In any case, the energy required to explain the light curve requires the ejecta to cool so slowly that the density will be too small for effective nucleation²³, and some observations support these ideas²⁴. The exclusion of type I's as nucleation sites is further strengthened by the suggestion (J. Scalo, in preparation) that sputtering by energetic electrons (Compton scattered by energetic ⁵⁶Co-decay γ rays or β^+ 's) will be sufficient to impede nucleation.

Grain temperature and hence predicted infrared fluxes would, of course, be sensitive to the presence of a radiative source underlying the mantle itself, but in the absence of clear indications of the requirement of such sources in SN II light curves^{10,12,25} we consider grain heating by inelastic collisions with thermal gas particles. The grain temperature θ can be estimated by balancing this heat input with the radiation losses of a grain²⁶,

$$\bar{Q}(\theta, a) \sigma \theta^4 \simeq 0.5 n \mu m_H v_{th}^3$$

where σ is the Stephan constant, v_{th} the gas thermal velocity, and the Planck mean absorption efficiency $\bar{Q} \ll 1$ for silicate grains in this size and temperature range²⁷. Because $\bar{Q}$ is small, θ does not differ markedly from the gas temperature $T_g(t)$. Fluxes at earth at various infrared wavelengths can then be predicted by assuming blackbody radiance of an equivalent sphere of radius vt at the grain temperature:

$$F_e \equiv \sigma \theta^4 b_\lambda \chi_\lambda (vt/d)^2,$$

$$b_\lambda \equiv \int_{\Delta\lambda} B_\lambda(\theta) d\lambda / \int_0^\infty B(\theta) d\lambda$$

is the fraction of Planck emission over $\Delta\lambda$ at temperature θ , d is the distance, and $\chi_\lambda \equiv \min(1, \tau_\lambda)$ is a correction for small optical depths. We consider the adiabatic expansion of a $3 M_\odot$ mantle,

with initial temperature, density and expansion velocity fit observationally inferred photospheric radii and effective temperatures¹⁵. Table 1 gives model characteristics and predicted broadband fluxes at Earth for the intervals 0.9–1.2 μm and 9.0–12.0 μm , for selected times in the first several years. No effect on the visual light curve is expected, as in the case of some novae²⁸, because of the absence of a strong underlying continuum source. Ground based detection of fluxes of 60 mJy are possible at 10 μm using a 60-inch telescope and an integration time of 10 min (A. Harper, personal communication). The predicted fluxes lie below this current limit for objects further away than about 10–20 kpc, implying that observations cannot provide conclusive tests of grain formation.

Grain survival in subsequent stages of SN remnant expansion up to encounter with the solar nebula is difficult to analyse without any clear model of such an interaction. Deceleration of the remnant as it sweeps up interstellar matter leads to the formation of a dense neutral shell with leading and trailing ionisation edges and associated ionising ultraviolet fluxes²⁹, and rarefaction (reverse shock) waves may produce observed 1–2 keV X-ray fluxes³⁰, but the radiation from such sources or heating by hot dilute remnant gas at these late stages has little disruptive effect on pre-existing grains³¹. Estimates of θ from radiative heating by the ultraviolet shell or thermal bremsstrahlung X-rays suggest values of only 10 K or so. If cosmic ray acceleration occurs in the same objects before nebula encounter, the possibility for electron sputtering exists (J. Scalo, in preparation), though this coincidence cannot at present be established.

Thus grains produced in such ejecta from a supernova near to the early Solar System seem likely to have survived until such time as they would encounter the primitive solar nebula. Details of an injection mechanism are unknown, and grain survival would depend on the extent of turbulence, mixing and clumpiness due to fluid instabilities like the Rayleigh–Taylor instability, caused by the supernova shock acting on the presupernova mantle or envelope itself (R. Chevalier, paper presented at UCSC Supernova Workshop), by the blast wave acting on the solar nebula before encountering the ejecta, or in the actual encounter of ejecta with the nebula (S.H.M., in preparation).

This work was supported in part by US NSF grants AST 74-21216 (University of Chicago) and AST 76-22673 (University of Illinois). S.W.F. thanks the R. R. McCormick Foundation and the Arthur Holly Compton Fund at the University of Chicago for support. The hospitality and stimulation of the 1977 UC Santa Cruz Supernova Workshop is also appreciated. We thank Gordon Lasher for helpful conversations.

SYDNEY W. FALK

Enrico Fermi Institute,
University of Chicago,
and

I.B.M.—T.J. Watson Research Center,
Yorktown Heights, New York

JAMES M. LATTIMER

Department of Astronomy,
University of Illinois,
Urbana, Illinois 61801

S. H. MARGOLIS

Enrico Fermi Institute,
University of Chicago,
933 E 56th Street,
Chicago, Illinois 60637

14. Arnett, W. D. *A. Rev. astr. Astrophys.* **11**, 73 (1973).
15. Kirshner, R. P. & Kwan, J. *Astrophys. J.* **197**, 415 (1975).
16. Hoyle, F. & Wickramasinghe, N. C. *Nature* **226**, 62 (1970).
17. Simons, S. & Williams, I. P. *Astrophys. Space Sci.* **39**, 123 (1976).
18. Arnett, W. D. *On the Bulk Yields of Nucleosynthesis From Massive Stars* (EFI Preprint 77-26, University of Chicago, 1977).
19. Anders, E. & Heymann, D. *Science* **164**, 821 (1969).
20. Howard, W. M., Arnould, M. & Truran, J. W. *Astrophys. Space Sci.* **36**, L1 (1975).
21. Colgate, S. A. & White, R. N. *Astrophys. J.* **157**, 623 (1969).
22. Falk, S. W. *Bull. Am. Phys. Soc.* **22**, 87 (1977).
23. Lattimer, J. M., Schramm, D. N. & Grossman, L. *Astrophys. J.* (in the press).
24. Friedjung, M. *Astr. Astrophys.* **34**, 419 (1974).
25. Falk, S. W. & Arnett, W. D. *Astrophys. J. Lett.* **180**, L65 (1973).
26. Falk, S. W. & Scalo, J. M. *Astrophys. J.* **202**, 690 (1975).
27. Gilman, R. C. *Astrophys. J. Suppl.* **28**, 397 (1974).
28. Clayton, D. D. & Hoyle, F. *Astrophys. J.* **203**, 490 (1976).
29. Chevalier, R. A. *Astrophys. J.* **188**, 501 (1974).
30. McKee, C. F. *Astrophys. J.* **188**, 335 (1974).
31. Burke, J. R. & Silk, J. *Astrophys. J.* **190**, 1 (1974).

Origin and nature of carbonaceous material in the galaxy

ASTRONOMERS generally believe that the carbonaceous material emerging from stars must be in the form of graphite, the most stable condensed form of carbon, and that such emergence must be confined to situations where the C/O ratio exceeds unity, such as in the atmospheres of carbon stars. We argue here that this state of affairs remains valid for mass flows from stars of sufficiently low surface temperatures, but it is not correct for low density flows from stars with colour temperatures $\geq 4,000$ K (or for oscillatory stars with colour temperatures that go above 4,000 K for a portion of their cycle). In the latter case we show that carbonaceous material comprised mainly of polysaccharides will be able to condense.

We have previously discussed the likely presence of polysaccharides in galactic infrared sources^{1,2}, and here we connect our discussion with the infrared model considered by Hoyle, Solomon and Woolf³. They considered that material flowed at rates $\sim 10^{-2} M_{\odot}$ per year from out of O stars of high mass. In such a model, radiation from the star is absorbed and re-emitted by the outflowing material, which produces a shielding of the true surface of the underlying star. The temperature of the material falls with increasing distance from the star, until at a stage where the temperature is in the range $\sim 5,000$ – $\sim 10,000$ K, an effective photosphere is formed. The effective photosphere develops at a radial distance $r_0 \sim 10^{14}$ cm from the star, where the hydrogen density $(n_H)_0$ is of order 10^{11} cm^{-3} (for a mass flow of $\sim 10^{-2} M_{\odot} \text{ yr}^{-1}$ at a speed of $\sim 300 \text{ km s}^{-1}$). Further out in the flow molecular condensations occur, leading to the eventual absorption of radiation emitted by the effective photosphere of the star, and to the re-radiation of the absorbed radiation in the infrared. Would the resulting infrared emission from the model of Hoyle *et al.*³ behave like the simpler polysaccharide models we considered previously^{1,2}? This is answered by Fig. 1, which shows the expected emission from the Hoyle *et al.*'s model compared to observations of the BN source^{4,5}. The expected emission was

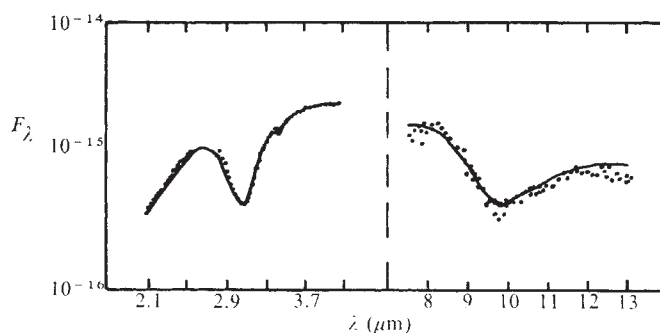


Fig. 1 Solid curve is the infrared emission for the source BN calculated for the model of Hoyle *et al.*³. The formation temperature of the polysaccharide grains was taken to be 850 K, and the grain temperature was taken to vary subsequently as the inverse square root of the distance from the exciting star. The optical depth of the region of formation of the grains was four times that of the sample of cellulose which gave rise to the transmittance values of Table 1 of ref. 2. The points represent observational data⁴.

Received 7 September; accepted 3 November 1977.

1. Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Astrophys. J. Lett.* **211**, L107 (1977).
2. Black, D. C. *Geochim. cosmochim. Acta* **36**, 377 (1972).
3. Clayton, R. N., Onuma, N., Grossman, L. & Mayeda, T. K. *Earth planet. Sci. Lett.* **34**, 209 (1977).
4. Podosek, F. A. & Lewis, R. S. *Earth planet. Sci. Lett.* **15**, 101 (1972).
5. Clayton, D. D. *Nature* **257**, 36 (1975).
6. Sabu, D. D. & Manuel, O. K. *Nature* **262**, 28 (1976).
7. Cameron, A. G. W. & Truran, J. W. *Icarus* **30**, 447 (1977).
8. Lattimer, J. M., Schramm, D. N. & Grossman, L. *Nature* (in the press).
9. Grasberg, E. K., Imshennik, V. S. & Nadezhin, D. K. *Astrophys. Space Sci.* **10**, 28 (1971).
10. Arnett, W. D. & Falk, S. W. *Astrophys. J.* **210**, 733 (1976).
11. Chevalier, R. A. *Astrophys. J.* **207**, 872 (1976).
12. Falk, S. W. & Arnett, W. D. *Astrophys. J. Suppl.* **33**, (1977).
13. Kirshner, R. P., Oke, J. B., Penston, M. & Searle, L. *Astrophys. J.* **185**, 303 (1973).

calculated for a polysaccharide formation temperature $T_m = 850$ K and for the relative transmittance values given in Table 1 of ref. 2. The radial opacity through the source was taken to be four times the relative values given in the latter table.

The new calculation is an improvement on our previous work in that no *ad hoc* assumption of a surface emitting like a black body has been made. This improvement, together with the agreement between theory and observation shown in Fig. 1, gives a strong empirical indication that polysaccharides can form in the mass flows from stars where the C/O ratio is probably less than unity. To give plausibility to such a view, three questions need answering: (1) Why is C not almost entirely consumed in the formation of CO? (2) Why is C not built into graphite rather than into polysaccharides? (3) In view of the inevitably small concentrations of the molecules C_2 , C_3 , how can the bulk of the carbon manage to condense at all?

The answer to question (1) turns on three points. The ratio CO/C at the effective photosphere is essentially thermodynamic, less than $\sim 10^{-6}$. For a constant outflow speed, the hydrogen density n_H falls off according to

$$n_H = (n_H)_0 (r_0/r)^2 \quad (1)$$

With $(n_H)_0 \approx 10^{11} \text{ cm}^{-3}$, and for a time scale of only a few years available for the recombination of atomic species into molecules, triple collisions of the kind $A + B + H \rightarrow AB + H$ play no significant role in the formation of diatomic molecules, which must accordingly proceed through two-body radiative recombinations of the type



With a low cross-section $\sim 10^{-22} \text{ cm}^2$ for $C + O \rightarrow CO + h\nu$ and for a gas thermal velocity $\sim 10^5 \text{ cm s}^{-1}$ the fraction CO/C built in the available time scale of 10^7 s is less than $\sim 10^{-2}$.

To answer question (2), we note that the internal energy of a large polyatomic molecule distributes itself statistically among the many states of the molecule, and it is appropriate to characterise the distribution by an internal temperature, T_m . The value of T_m for a particular molecule is determined by the equation of energy balance between the absorption of radiation from the star and the re-emission of radiation by the particular molecule, an equation of the form

$$(r_0/r)^2 \int_0^\infty Q_{\text{abs}}(\lambda) B_\lambda(T) d\lambda = 4 \int_0^\infty Q_{\text{em}}(\lambda) B_\lambda(T_m) d\lambda, \quad (3)$$

where B_λ is the Planck function. The factor 4 is for spherical particles, and it assumes no radiation field in the infrared. The presence of an infrared field reduces the factor 4, in a typical case to ~ 2 . This reduction has no significant effect on the above argument since the reduction would apply to both the pyran ring and the graphite ring. Both the C_6 ring which appears in graphite and the C_5O pyran ring which appears in the commonest polysaccharides absorb mainly in the ultraviolet with comparable values of Q_{abs} . But the pyran ring has a much higher value of Q_{em} in the infrared than the C_6 ring, because the latter is a symmetric molecule with no dipole moment. Hence equation (3) leads to a significantly higher value of T_m for C_6 than it does for C_5O , and this higher value of T_m more than offsets the greater binding energy of the C_6 ring. (The binding energy difference is not large. The binding of the C_6 ring from its constituent atoms is 30.5 eV and that for the C_5O ring is ~ 29 eV (refs 6, 7).)

For question (3) begin by assuming the initial existence of a single polymer chain which we take to have been built through C_2, C_3 . The logic will be that this first polymer chain, through rapid building interspersed by repeated fragmentation, can generate a vast cascade of further polymer chains which have nothing to do with the low concentrations of C_2, C_3 . The logic is similar to that of the explosion of a nuclear weapon, where a first neutron is enormously amplified by the fission cycles which it provokes, and

where the flood of subsequent neutrons have nothing to do with the source of the first neutron.

For a flat chain of width D , length l , the number of carbon atoms arriving in a time dt which could lead to chain growth is

$$dN \approx 2 n_c \langle v_{\text{th}} \rangle l D dt \quad (4)$$

[With $n_0 \gtrsim n_c$, the oxygen rate is moderately larger than equation (4).] Further, assuming a mean length interval between two successive polymer rings to be about $2D$ and with six atoms making up a ring the increment of length corresponding to dN is

$$dl \approx f (dN/6) 2D = (D dN/3) f, \quad (5)$$

where f is the fraction of impinging carbon atoms that diffuse and attach themselves to the ends of the polymer. Equations (4) and (5) give

$$l = l_0 e^{t/\tau_1} \quad (6)$$

with

$$\tau_1 = \left[\frac{2}{3} n_c \langle v_{\text{th}} \rangle D^2 f \right]^{-1}. \quad (1)$$

The phenomenon of exponential growth we describe here is the same as that which occurs in the building of 'whiskers', which has been studied in the laboratory^{8,9}. For $n_c \approx 10^6 \text{ cm}^{-3}$, $D \approx 5 \times 10^{-8} \text{ cm}$ and $\langle v_{\text{th}} \rangle \approx 10^5 \text{ cm s}^{-1}$, therefore we obtain $\tau_1 \approx 2 \times 10^3 f^{-1} \text{ s}$, and this is small compared to the available time scale of the order of a year, even for fractions f as low as 10^{-2} . Ample time is therefore available for many exponential 'cycles', again in analogy to the many fission cycles of a nuclear weapon.

We expect polymers to appear in the mass flow from a star when the temperature T_m first becomes low enough for the bond linkages in the polymer to assume stability. For C–O–C linkages of a polysaccharide, the bond strength is ~ 4 eV and the largest values of T_m for which such a bond will be stable lies in the range 800–900 K, just the polysaccharide formation temperature used in the calculations leading to Fig. 1.

The first polymers built through C_2, C_3 , will be of short lengths and for them the value of T_m determined by equation (3) is less than for longer polymers with lengths $l \approx \lambda_{\text{star}}/2\pi$. This is because a tuning effect appears as the polymers grow to a length that is resonant with the main optical radiation of the star, $\lambda_{\text{star}} \approx 5 \times 10^{-5} \text{ cm}$. The polymers then experience a strong radiation pressure force due to scattering which gives them an appreciable drift velocity with respect to the ambient gas. Hoyle *et al.* estimated a drift velocity between 10 and 100 km s⁻¹ for their model. At 100 km s⁻¹ the viscous drag would raise the temperature of the polymer chain by about 100 K. We think even stronger heating than this could occur. Yet even 100 K is sufficient in a marginally stable situation to begin breaking the C–O–C linkages of the polymer. The reduced lengths of the fragments destroys the resonance, so that the fragments quickly assume lower values of T_m , thereby returning to a stable condition. Because of the explosive exponential growth implied by equation (6), the fragments almost immediately go through the same sequence as the original polymer. Not only do the lengths of the polymers grow exponentially but the numbers of the polymers also increase dramatically.

There is, of course, a limit to the number of particles that can be produced from a single first polymer chain, but the limit turns out to be very large. The limit arises because a first polymer chain, together with all its progeny, does not have access to all the carbon present in the whole mass flow. But there will be access at least to all the carbon within the distance through which carbon atoms can diffuse in the time scale of $\sim 10^8$ s required for a given sample of material to flow from the star out to distances at which the density

falls off significantly. Thus the carbon diffusion distance in a time t is given by

$$\sim [\langle v_{th} \rangle t / \sigma_c n_H]^{1/2}$$

where σ_c is the cross-section for collisions between carbon atoms and hydrogen atoms. With $t \approx 10^8$ s, $\langle v_{th} \rangle \approx 10^5$ cm s⁻¹, $\sigma = 10^{-16}$ cm², $n_H \approx 10^{10}$ cm⁻³, the diffusion distance is $\sim 3 \times 10^9$ cm. Thus the zone of influence of an initial polymer is at least $(3 \times 10^9)^3$ cm³. It is actually greater than this because the polymers are subject to radiation pressure which causes a relative motion in the radial direction with respect to the gas. Using the relative velocity, 100 km s⁻¹ by Hoyle *et al.* the radial drift with respect to the gas developed by a polymer in the time scale $t \sim 10^8$ s is as large as 10^{15} cm. Thus the zones of influence of initial polymers are cylindrical in shape with axes of length $\sim 10^{15}$ cm along the radial direction from the star and with transverse dimensions of $\sim 3 \times 10^9$ cm, the latter being the carbon atom diffusion distance calculated above. The zone of influence of a single starting short polymer chain, therefore, has volume of order $10^{15} (3 \times 10^9)^2 \approx 10^{34}$ cm³, compared with a total volume of $\sim 10^{45}$ cm³ for the effective condensation region of the whole mass flow. Only some 10^{11} starting polymers are needed to mop up the whole of the carbon, 10^{11} starting polymers in 10^{45} cm³ giving a space density of 10^{-34} cm⁻³.

The required formation rate through C_3, C_3 of initial short polymer chains therefore takes the exceedingly low value of $\sim 10^{-38}$ cm⁻³ s⁻¹. The rates of formation of C_3, C_4 by $C_2 + C \rightarrow C_3$, $C_3 + C \rightarrow C_4$ are uncertain, but even with low cross-sections for both reactions, and even with ample allowance for the destruction of C_2 through $C_2 + O \rightarrow CO + C$, a fraction $\sim 10^{-12}$ of all the carbon will become C_4 in the available time scale. The molecule C_4 already has a sufficient number of internal states for further additions to occur with comparatively large cross-sections, $\sim 10^{-16}$ cm². Thus beyond C_4 the apparent difficulty of small formation rates disappears, and the number of initial polymer chains that could be achieved (if necessary) could be comparable to the number of C_4 nuclei, which is very many orders of magnitude greater than the minimum required number of initial short polymer chains. The first such chains to flow through $C_2, C_3, C_4 \dots$ therefore go on to take the whole of the carbon. This completes the answer to the third question.

If mass loss from a highly luminous O star proceeds for long enough, the oxygen-rich envelope will be replaced by material that has been processed in the star by the CN cycle. The oxygen will now be almost totally depleted, the carbon abundance will be reduced to about 1/10 of its normal value, and there will be a large excess of nitrogen. Under these conditions C_4 rings would evolve into heterocyclic C_4N , C_5N and C_4N_2 rings. It may well be important in this connection that the porphyrins have a strong absorption band near 4,430 Å, the wavelength of a well-known interstellar absorption feature¹⁰, and that quinazoline and its derivatives have a strong absorption at 2,200 Å (see ref. 11) close to another interstellar feature.

Photospheric temperatures of evolved supergiant stars are uncertain. If mass flows in M and N-type stars originate in layers of the atmosphere where the colour temperature $> \sim 4,000$ K, arguments similar to those discussed above would apply.

We conclude the following. Infrared sources exhibiting polysaccharide absorption features may be associated with massive stars of the kind discussed by Hoyle *et al.*³. Mass flows from such stars can lead to the production of polysaccharides in the first instance, followed by the condensation of nitrogenated heterocyclic carbon compounds. The composition of carbonaceous condensates from these sources may well determine the course of interstellar chemistry. Low molecular weight organic compounds and radicals detected in interstellar clouds, often involving a number of linked carbon atoms, are probably the products of the ultraviolet and cosmic-ray degradation of much more complex structures.

We also speculate that carbonaceous materials formed in the mass flows from stars provided the chemical basis for the origin of

life. It is not as hard as it has usually been supposed to understand how interstellar materials could have by-passed the destructive high temperature of the solar nebula and could have arrived undamaged at the Earth. We suggest that the origin of life on the Earth involved essentially the assembly of complex chemical components which had already been provided in the last stages of the accumulation of the Earth, and which exist widely and in comparatively great quantities within the interstellar medium.

We thank Professor P. M. Solomon for helpful discussions.

F. HOYLE*

N. C. WICKRAMASINGHE

Department of Applied Mathematics and Astronomy,
University College,
P.O. Box 78,
Cardiff, UK

Received 9 August; accepted 13 October 1977.

*Honorary Professorial Fellow. Present address: Cockley Moor, Dockray, Penrith, Cumbria.

1. Hoyle, F. & Wickramasinghe, N. C. *Nature* **268**, 610-612 (1977).
2. Hoyle, F. & Wickramasinghe, N. C. *Mon. Not. R. astr. Soc.* **181**, 51P (1977).
3. Hoyle, F., Solomon, P. M. & Woolf, N. J. *Astrophys. J.* **185**, L89 (1973).
4. Gillett, F. C. & Forrest, W. J. *Astrophys. J.* **179**, 483 (1973).
5. Merrill, K. M., Russell, R. W. & Soifer, B. T. *Astrophys. J.* **207**, 763 (1976).
6. Cox, J. D. *Tetrahedron* **19**, 1175 (1963).
7. Dewar, M. J. S. & Harget, A. J. *Proc. R. Soc. Lond. A* **315**, 442 (1970); **315**, 457 (1970).
8. Meyer, L. *Proc. 3rd Conf. Carbon* 451. (Pergamon Press, New York, 1959).
9. Donn, B. D. & Sears, B. W. *Science* **140**, 1208 (1963).
10. Johnson, F. M. *Ann. N.Y. Acad. Sci.* **194**, 3 (1971).
11. Albert, A. & Armarego, W. L. F. *Adv. Heterocyclic Chem.* **4**, 1 (1965).

ELF emissions observed at Moshiri

MORPHOLOGICAL characteristics of ELF emissions (frequency 2-3 kHz) observed at a low latitude ground station of Moshiri in Japan (geomag. lat. 34.5°N; $L = 1.59$) are reported here. ELF emissions in this frequency range are most poorly understood in the magnetospheric wave phenomena, and the information obtained on the ground will be of crucial importance in the study of their generation and propagation mechanisms. We have been observing VLF and ELF emissions at Moshiri¹⁻³ since 1964. We believe that Moshiri is at a lower latitude than any other where the emissions are continuously recorded⁴. Emissions of the hiss type are measured, by the minimum detection method¹, at four specific

Fig. 1 Sonagrams of ELF emissions observed at Moshiri. *a*, 0950 LT 11 January 1976; *b*, 0750 LT 2 April 1976.

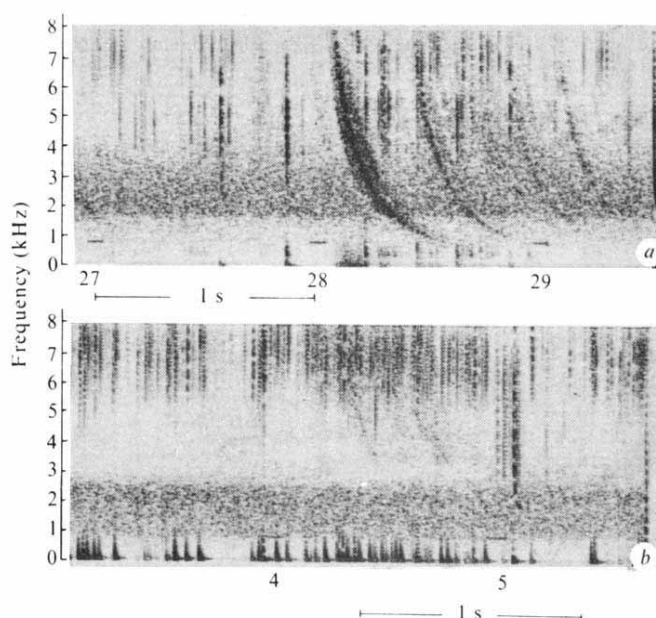
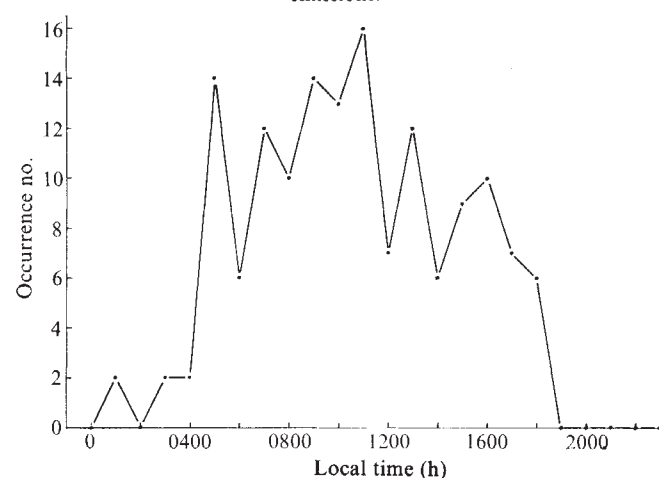


Table 1 Characteristics of ELF emissions

	Ground observations	Satellite observations	
		Pogo (Kimura)	Isis (Ondoh)
Period of data	January 1974–December 1976	July 1968–May 1969	July 1970–April 1971
Diurnal variation	5–18 h LT	3–12 h LT	?
K_p dependence	$K_p > 4$	higher K_p	$K_p = 1-3$
Frequency spectrum	2–3 kHz mainly	2 ± 0.5 kHz	< 3 kHz
Latitudinal distribution	Tweek cutoff: Yes, high latitude penetration No, low latitude penetration	Peak at high latitudes Wide latitude coverage	Peak at 50–60° Wide latitude coverage
K_p LT dependence	Shift to morning with increasing K_p	?	?
K_p -frequency spectrum	No relation	?	Wider band with increasing K_p
LT-frequency spectrum	No relation	?	?

frequencies of 8 ($\Delta f = \pm 1.25$ kHz), 5 (± 1 kHz), 1.5 (± 0.1 kHz) and 0.8 (± 0.1 kHz) kHz, and recorded on paper charts. Wide band (0–10 kHz) observations on magnetic tapes have also been conducted following the synoptic schedule of two minutes every hour. We have found, using Ariel 3 VLF data, that the storm-time VLF emissions at Moshiri are generated around the plasmopause by the electron cyclotron instability with ring current electrons⁵. But the large number of VLF emissions appearing during magnetically quiet times have not been well studied². Satellite measurements made on board Pogo (I. Kimura, personal communication) and Isis⁶ have yielded a new type of ELF emissions ($f < 3$ kHz).

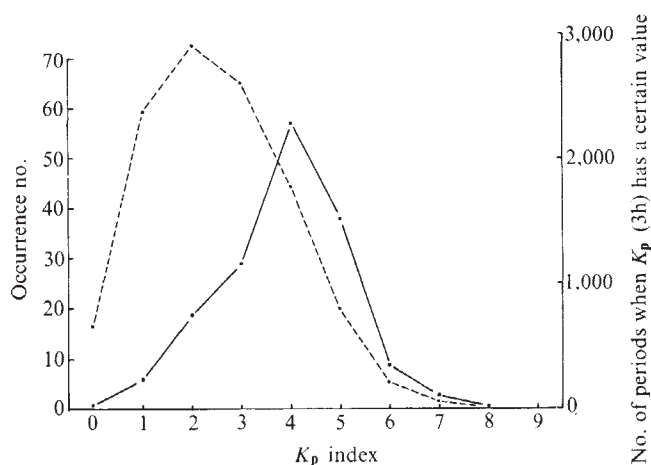
Here we describe the morphology of those ELF emissions observed at Moshiri based on magnetic tape records during four years from January 1973 to December 1976. During this period we identified about 160 ELF emission events. They were mainly of the hiss type, but we could find others including the chorus structure. Moshiri is located in an isolated village, free from artificial noises, and we could not detect any fine structure due to the power line harmonics radiation^{7,8}. Hence, we consider that these ELF emissions are a natural phenomenon. Figure 1 shows the frequency spectra of ELF emissions; *a* is the sonogram of typical narrow band ELF emissions whose energy is concentrated in 2–3 kHz, and this frequency band is most often seen, whereas *b* shows lower frequency emissions (1–2.5 kHz). The low frequency

Fig. 2 Local time dependence of occurrence number of ELF emissions.

cutoff frequency of ELF emissions is, on occasions, coincident with the cutoff frequency of the first order mode of the Earth-ionosphere waveguide propagation or tweeks. Examination of these sonagrams suggests that the frequency band of 2–3 kHz depends neither on local time nor K_p index, which is in good agreement with satellite results⁹.

Figure 2 shows the local-time dependence of the occurrence number, and indicates that these ELF emissions occur predominantly in the daytime (5–18 h LT). A comparison of local-time dependences for three ranges of K_p index shows that the occurrence number is concentrated in the afternoon during quiet periods $K_p \leq 2+$, but in the morning during moderate disturbances $3- \leq K_p \leq 5+$. Figure 3 shows the K_p dependence of occurrence number of ELF emissions (full line), and the number of periods when K_p (3 h) has a certain value (broken line). A comparison of the two curves indicated that the occurrence probability shows an abrupt increase from $K_p = 4$, and a broad maximum in the K_p range from 5 to 7, in good agreement with the satellite result. So, ELF emissions are likely to be associated with magnetic disturbances.

Assuming that the propagation under the ionosphere is roughly confined to the magnetic meridian plane⁹, ELF emissions with a low frequency cutoff, being associated with the tweeks cutoffs, are considered to have penetrated the ionosphere at latitudes higher than the station latitude, and followed the Earth-ionosphere waveguide mode of propagation¹⁰. There are also ELF emissions

**Fig. 3** Dependence on K_p index of occurrence number of ELF emissions and the number of periods in which K_p has a certain value.

whose lower cutoff frequencies extend well below the cutoff for the Earth-ionosphere waveguide mode propagation (see Fig. 1b). This indicates that these emissions seem to have emerged from the ionosphere near the station, with their wave normal directions in the ionosphere lying within the transmission cone¹¹. These characteristics are not incompatible with the wide latitudinal coverage found by the satellites⁶. Table 1 summarises the general properties of ELF emissions based on our ground observations and on satellite results. Our initial impression is that these properties are similar to those of plasmaspheric hiss whose centre frequency is much lower, ~ 0.5 kHz¹²⁻¹⁴.

The generation and propagation mechanisms of ELF emissions need further study. Their narrow band nature, however, suggests that the generation region may be very localised in the equatorial plane⁵. Considering that their occurrence is highly enhanced at 50–60° (ref. 6), the generation possibly occurs just within the plasmopause. If the waves are generated at various wave normal angles, some can propagate in complex non-ducted paths to the ionosphere over a wide latitude range⁶ and, for favourable wave normal directions¹¹, can be detected on the ground. The association of the occurrence of ELF emissions with magnetic storms or substorms is being investigated, and could give us insight into the

energetic particles in the outer radiation belt responsible for the excitation of ELF emissions.

MASASHI HAYAKAWA
YOSHIHITO TANAKA

Research Institute of Atmospherics,
Nagoya University,
Toyokawa, Aichi, 442, Japan

Received 26 July; accepted 28 October 1977.

1. Iwai, A., Otsu, J. & Tanaka, Y. *Proc. res. Inst. Atmos.* **11**, 29–40 (1964).
2. Tanaka, Y., Hayakawa, M. & Ohtsu, J. *Rep. ionosph. Space Res.* **28**, 168–172 (1974).
3. Hayakawa, M., Tanaka, Y. & Ohtsu, J. *J. atmos. Terr. Phys.* **37**, 517–529 (1975).
4. Jorgensen, T. S. *J. geophys. Res.* **71**, 1367–1375 (1966).
5. Hayakawa, M., Bullough, K. & Kaiser, T. R. *Planet. Space Sci.* **25**, 353–368 (1977).
6. Ondoh, T., Aikyo, K. & Nagayama, M. *J. Radio Res. Labs. Jap.* **19**, 23–51 (1972).
7. Helliwell, R. A., Katsufakis, J. P., Bell, T. F. & Raghuram, R. *J. geophys. Res.* **80**, 4249–4258 (1975).
8. Bullough, K., Tatnall, A. R. L. & Denby, M. *Nature* **260**, 401–403 (1976).
9. Iwai, A. & Tanaka, Y. *Proc. res. Inst. Atmos.* **15**, 1–16 (1968).
10. Otsu, J. *Proc. res. Inst. Atmos.* **7**, 58–71 (1960).
11. Hayakawa, M. & Iwai, A. *J. atmos. Terr. Phys.* **37**, 1211–1218 (1975).
12. Thorne, R. M., Smith, E. J., Burton, R. K. & Holzer, R. E. *J. geophys. Res.* **78**, 1581–1596 (1973).
13. Thorne, R. M., Church, S. R., Malloy, W. J. & Tsurutani, B. T. *J. geophys. Res.* **82**, 1585–1590 (1977).
14. Bullough, K. *et al.* *Proc. R. Soc. Lond.* **A343**, 207–226 (1975).

A vitreous Kr₂SiO₂

THERE are many studies^{1–7} of the presence of inert gases in glasses. Because they have no electron affinity and high ionisation potentials (12 eV for Xe to 24 eV for He) the solubility of inert gases in glasses has been considered a simple physical process with no strong chemical interaction within the glass network. Recent work on pressure dependence of inert gas in vitreous¹ and crystalline² silica (SiO₂) showed that the equilibrium solubility may be explained qualitatively by a statistical thermodynamic model³. At non-equilibrium conditions, however, a large quantity of inert gases can be incorporated into SiO₂ by processes such as glow discharge^{8,9} and sputtering^{10,11}, and here, the amount of inert gases in SiO₂ far exceeds the equilibrium solubility. Using a high-rate sputtering technique, we prepared a SiO₂ deposit containing 16.1 at.% Kr, expressed by a

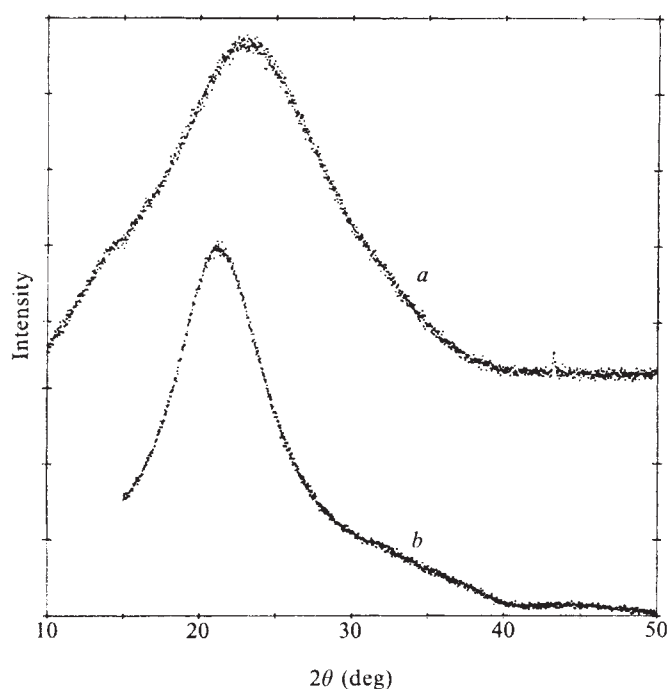


Fig. 1 X-ray diffraction patterns of the Kr₂SiO₂ deposit (a) and the target SiO₂ (b).

glass formula, Kr₂SiO₂ and we report here the unique characteristics of this material.

The Kr₂SiO₂ deposit was prepared by a modified r.f. sputtering apparatus^{12,13} at a sputtering rate of 30 μm^{−1}h. The target was a 12.7-cm-diameter, 0.64-cm-thick fused SiO₂ disk. Sputtering gas was krypton and oxygen 1 : 1 mixture; the addition of oxygen to the sputtering gas was required for stoichiometric-oxide formation. Deposition was onto a water-cooled copper substrate that had floating electrical potentials.

The Kr₂SiO₂ deposit was about 0.2 mm thick, and had the general appearance of a thin fused silica plate. Its

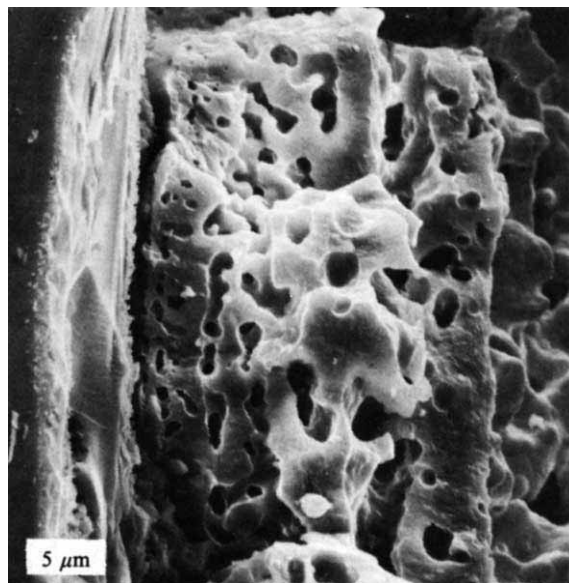


Fig. 2 Formation of the micropores after heating the Kr₂SiO₂ deposit to 1,000 °C.

surface was smooth, and contained a low density of macroscopic defects under ×100 magnification. A fracture surface along the thickness of the deposit resembled the fracture of an oxide glass.

The compositions of Kr and Si were determined by non-dispersive X-ray fluorescence analysis. The X-ray intensity of Kr was corrected for self-absorption assuming a uniform distribution of Kr throughout the deposit. It indicated a Kr/Si mole ratio of 0.57 ± 0.066, or 16.1 at.% Kr, from which the Kr₂SiO₂ formula was assigned.

By comparison with the X-ray diffraction pattern of the fused SiO₂ target the diffraction peak of the Kr₂SiO₂ deposit was nearly two times broader (Fig. 1). This indicated an increasingly disordered short-range atomic packing in the Kr₂SiO₂ deposit, possibly due to combined effects of high-rate sputtering and Kr entrapment, but the Kr₂SiO₂ had high thermal stability based on no weight loss observed after isothermal treatments at 700 °C for 2 h in air. Rapid release of Kr was observed during 1,000 °C annealing, and the sample became a popcorn-like flake containing 0.1 to 10 μm size pores (Fig. 2).

This may be the first time that such a high content of Kr has been incorporated into SiO₂. It would be interesting to consider the role of Kr in the structure. Because the general appearance and the properties of the deposited Kr₂SiO₂ are similar to fused silica in many aspects¹³, we can assume that Kr atoms participate little in bonding with the network atoms, but are trapped in the vitreous SiO₂. If Zachariasen's glass network model is assumed for the SiO₂ structure, we can see that those pores made by 4–7 oxygen atoms are too small to accommodate the Kr atom which has an atomic diameter of 3.99 Å and an atomic volume of 44.95 Å³ at 0 K (ref. 14). As shown in Fig. 3,

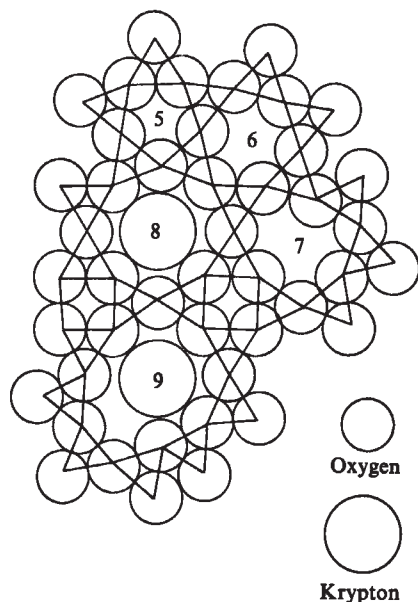


Fig. 3 Possible trapping of single Kr atom in the SiO_2 network. Different pore sizes are shown by the number of oxygen atoms.

only pores made by eight or more oxygen atoms are big enough to trap the Kr atom. Since there are few such large pores (probably 10%) in the homogeneous structure model, the number of Kr atoms trapped cannot account for the Kr composition. We may consider two possible mechanisms for the Kr entrapment: (1) the $\text{Kr}\cdot 2\text{SiO}_2$ consists of an SiO_2 network similar to that of fused SiO_2 ; a portion of Kr atoms is trapped individually in the pores made by eight or more oxygen atoms and a portion of Kr atoms is in the form of inhomogeneous clusters, and (2) the $\text{Kr}\cdot 2\text{SiO}_2$ is composed of a distorted SiO_2 network to accommodate individual Kr atoms.

I thank N. Laegreid, R. W. Moss, M. A. Bayne and E. D. McClanahan for preparing the sample by high-rate r.f. sputter deposition and K. Nielson for making the X-ray fluorescence analysis. This work is sponsored by the US ERDA.

RONG WANG

Materials Department,
Battelle Pacific Northwest Laboratory,
Richland, Washington 99352

Received 14 September; accepted 25 October 1977.

- Shelby, J. E. *J. appl. Phys.* **47**, 135 (1976).
- Barrer, R. M. & Vaughan, D. E. W. *Trans. Faraday Soc.* **63**, 2275 (1967).
- Shackelford, F. J., Studt, R. L. & Fulrath, R. M. *J. appl. Phys.* **43**, 1619 (1972).
- Doremus, R. H. in *Glass Science*, ch. 8 (Wiley, New York, 1973).
- Shelby, J. E. *J. Am. ceram. Soc.* **55**, 61 (1972).
- Shelby, J. E. *Phys. Chem. Glasses* **13**, 167 (1972).
- Swets, D. E., Lee, R. W. & Frank, R. C. *J. chem. Phys.* **34**, 17 (1961).
- Grant, W. A., Carter, G. *Vacuum* **15**, 477 (1965).
- Young, J. R. *J. appl. Phys.* **27**, 926 (1956).
- Hoffmeister, W. & Zuegel, M. *Thin Solid Films* **3**, 35 (1969).
- Petersson, S., Linker, G. & Meyer, O. *Phys. Stat. Sol. a* **14**, 605 (1972).
- McClanahan, E. D., Busch, R., Fairbanks, J. & Patten, J. W., presented at Gas Turbine Conference and Products Show, Zurich, Switzerland, March 3–April 14, 1974.
- Wang, R. & Bayne, M. A., *Proceedings of Second American Nuclear Society Meeting on Fusion Technology*, p. 447 (1976).
- Donohue, J. *The Structure of Elements* ch. 2, 27 (John Wiley, New York, 1974).

Anion deficiency in strontium titanate

STUDIES of the anion deficient phases of titanium and vanadium perovskites showed that the perovskite SrTiO_{3-x} has a wide range of composition extending from $x = 0$ to $x = 0.5$ (ref. 1). The nonstoichiometry was inferred from the monophasic behaviour of the solid within this compositional range as obtained by means of powder X-ray diffraction. The same cubic structure was found for both $\text{SrTiO}_{2.5}$ and SrTiO_3 . Perovskites have been widely

studied recently in view of the very interesting and useful properties of these oxides². The physicochemical properties of SrTiO_3 include semiconductivity^{3,4} and superconductivity⁵ as well as peculiar phase transitions⁶. Of particular interest in this, and other⁷, systems is the presence of point defects and their influence on those properties. It seems that, at small degrees of reduction, doubly ionised oxygen vacancies are the main defects present on SrTiO_{3-x} (ref. 8). Although in that case, the oxygen vacancies, being at small numbers, are probably distributed at random, the presence of so many empty oxygen positions as the stoichiometry of $\text{SrTiO}_{2.5}$ implies—one in every six oxygens missing—automatically suggests the possibility of ordering. This possibility seems to be realised under the synthesis conditions that we have used and we report here some of the results so far obtained in the system $\text{SrO-Ti}_2\text{O}_3\text{-TiO}_2$.

Samples of composition SrTiO_{3-x} with several values of x were prepared by heating appropriately weighted amounts of the parent oxides in quartz ampoules, sealed under vacuum, at 1,200 °C for 2 weeks. The ampoules were then quenched in liquid nitrogen. We have never obtained a homogeneous product but rather a compact powder with colours ranging from violet to yellow from the outside to inside. The X-ray data of a blue sample of nominal composition $\text{SrTiO}_{2.5}$ could be interpreted as from a cubic unit cell with $a = 3.900 + 0.003$ Å, in excellent agreement with the previously accepted value¹. No superstructure lines were detected. Electron diffraction showed a very different situation, however. Figure 1 shows a pattern obtained on a Siemens 102 Elmiskop microscope fitted to a double tilting stage and operated at 100 KV. This pattern can be indexed as from the $[\bar{1}\bar{2}\bar{1}]$ zone axis of the same cubic subcell. There is a clear sixfold superlattice along $\bar{1}11$ suggesting a long spacing of ~ 13.52 Å. Figure 2 shows an electron micrograph of the same crystal area where the super-lattice fringes are resolved.

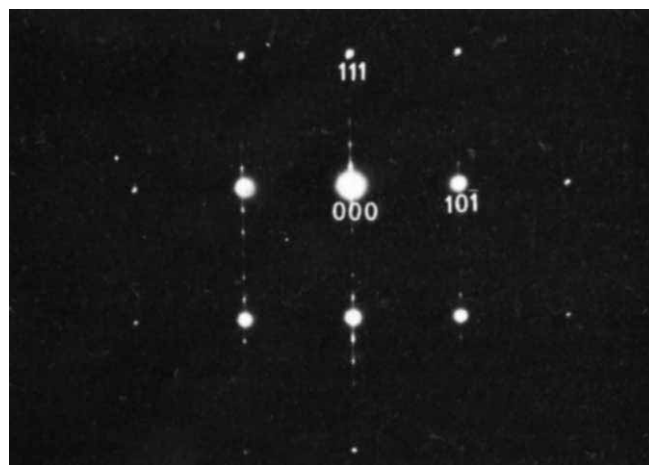


Fig. 1 Electron diffraction pattern of $\text{SrTiO}_{2.5}$ showing a sixfold superlattice along $\bar{1}11$ of the perovskite subcell. Zone axis $[\bar{1}\bar{2}\bar{1}]$.

The diffraction pattern is faintly streaked and it can be seen that the fringes are not equidistant, although their separation is often of ~ 13 Å. On this particular sample the superlattice was always sixfold and lying on $\bar{1}11$. Figure 3 shows an even more interesting example. This pattern, from another crystal of the same blue powder, can be indexed on the $[1\bar{1}0]$ zone axis of the cubic subcell. It can be seen that there are in fact two sixfold superstructures lying along $\bar{1}11$ and $\bar{1}\bar{1}\bar{1}$. Again there is streaking along those two directions. Although at first sight this pattern could be attributed to a twinned crystal the situation is somewhat more complicated as shown on the corresponding micrograph, Fig. 4. It can be seen that there are two sets of lattice fringes which cross at an angle of 70.5° as expected from the diffraction pattern. Even more interesting is the fact that the fringes intercross without apparent distortion and also that they are not always perfectly straight. On the other hand some of them, especially the set shown at B do end within the crystal and not at the crystal edge. The



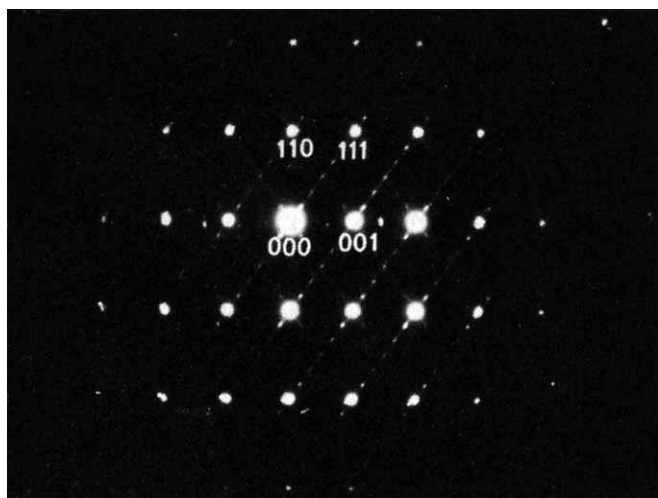
Fig. 2 Electron micrograph corresponding to Fig. 1 showing the superlattice fringes. Note that the fringe spacing is irregular.

Scale bar, 100 Å.

streaking is clearly understood in view of the uneven separation between the fringes in both sets.

The removal of an oxygen ion from a close-packed structure produces a drastic symmetry change in the environment of the metallic atom (or atoms) as in this case, coordinated to the oxygen. At the limit, this can produce a change in the coordination geometry around those metallic atoms, if they are capable of existing in more than one kind of coordination. In such cases one can no longer speak of anion vacancies in the usual sense of point defects since they are elements of the structure which are just unoccupied⁹. These subtle changes in coordination are difficult to detect unless they are ordered¹⁰ and it is not uncommon in the anion-deficient perovskite for an ordered

Fig. 3 Electron diffraction pattern of $\text{SrTiO}_{2.5}$ showing two sixfold superlattices along $\bar{1}11$ and $\bar{1}\bar{1}\bar{1}$ of the perovskite subcell. Zone axis $[110]$.



arrangement of vacancies¹¹ to be found. This is the case for $(\text{Ba}, \text{Sr})\text{MnO}_{3-x}$ (ref. 12) and BaFeO_{3-x} (ref. 13) where the Mn^{3+} and Fe^{3+} ions are ordered having bipyramidal-trigonal and tetrahedral coordination respectively. But, as Ti^{3+} is not a typical ion, adopting coordination geometries other than octahedral, we cannot yet give a structural model to explain this superperiodicity. However, the evidence shown above strongly suggests that in the synthesis conditions that we used, anion deficiency in SrTiO_{3-x} can be accommodated by planar defects parallel to (111)

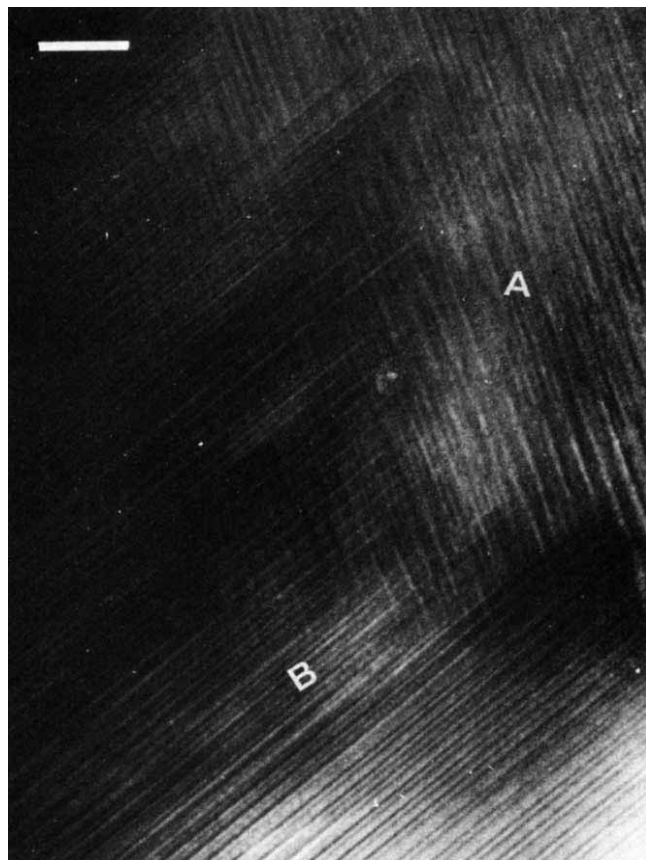


Fig. 4 Electron micrograph corresponding to Fig. 3. Note that the superlattice fringes intercross without apparent distortion and that many of them, especially those of set B, end within the crystal. Scale bar, 50 Å.

of the cubic subcell. In other words, there seems to be some kind of long-range order of the empty anion positions. It can also be expected that with different separations between these planar defects a family of new ordered phases, based on the perovskite structure, can be obtained. Furthermore, since we have commonly found two sets of crossing extended defects, it could well be that a new kind of block-type structures, analogous to those formed by double crystallographic shear on ReO_3 -type oxides, can be formed based on the perovskite subcell. Experiments are now in progress to test this possibility and also to determine to what extent planar defects affect the properties of strontium titanate.

We thank L. Puebla and A. Garcia for technical assistance.

M. A. ALARIO FRANCO
M. VALLET REGÍ

Departamento de Química Inorgánica,
Facultad de Ciencias Químicas,
Universidad Complutense,
Ciudad Universitaria,
Madrid-3, Spain

Received 26 September; accepted 26 September 1977.

*Also at Instituto de Química Inorgánica Elhuyar C.S.I.C. Serrano, 113 bis. Madrid, Spain.

1. Kestigian, M. Dickinson, J. G. & Ward, R. J. *Am. chem. Soc.* **5**, 5598-5601 (1957).
2. Goodenough, J. B. & Longo, J. M. *Crystallographic and Magnetic Properties of Perovskite and Perovskite Related Compounds*. Landolt. Börnstein New Series, Group III, Vol. 4a. New York: Springer Verlag (1970) p 126-314.

3. Cardona, M. *Phys. Rev.* **140**, A651–A655 (1965).
4. Lee, C., Yahia, J. & Brebner, J. L. *Phys. Rev.* **B3**, 2525–2533 (1971).
5. Koonce, C. S., Cohen, M. L., Schooley, J. E., Hosler, W. R. & Pfeiffer, E. R. *Phys. Rev.* **163**, 380–390 (1967).
6. Chang, T. S., Holzkichter, J. S., Jmbusch, G. F. & Schawlow, A. L. *Solid State Commun.* **8**, 1179–1181 (1970).
7. Anderson, J. S. in *Surface and Defect Properties of Solids* (eds Roberts, M. W. & Thomas, J. M.) **1**, 1–53 (Chemical Society, London, 1972).
8. Yamada, H. & Miller, G. R. *J. Solid State Chem.* **6**, 169–177 (1973).
9. Anderson, J. S. in *Modern Aspects of Solid State Chemistry* (ed. Rao, C. N. R.) **29**–106 (Plenum, New York, 1970).
10. Gorter, E. W. *Proc. Intern. Congr. Pure and Appl. Chem.* **17th Congr.** **1**, 300–306 (1959).
11. Negas, T. & Roth, R. S. *Proc. 5th Materials Research Symp. Solid State Chem. N.B.S. Spec. Publ.* **364**, 233–263 (1972).
12. Jacobson, A. J. & Horrox, A. J. W. *Acta Crystallogr.* **B32**, 1003–1008 (1976).
13. Jacobson, A. J. *Acta Crystallogr.* **B32**, 1087–1090 (1976).

Climatic information from $^{18}\text{O}/^{16}\text{O}$ analysis of cellulose, lignin and whole wood from tree rings

MEASUREMENTS of stable isotope ratios on tree rings have proved a promising way of determining past climate^{1–5}. Recent discussions have centred on which component of wood provides the most reliable record^{6,7}. We have measured the oxygen isotopic composition of cellulose, whole wood and lignin from tree rings in a white spruce (*Picea glauca*) which grew in the Edmonton area from 1882 to 1969. Using meteorological records we have evaluated the responses of the $^{18}\text{O}/^{16}\text{O}$ ratios of the three components of the tree rings to seasonal temperatures. Results show a high correlation between cellulose $^{18}\text{O}/^{16}\text{O}$ ratios and mean annual temperature, a poorer, though still significant correlation between whole wood $^{18}\text{O}/^{16}\text{O}$ ratios and mean annual temperature and no significant correlation between lignin $^{18}\text{O}/^{16}\text{O}$ ratios and mean annual temperature.

Whole wood measurements were made on 5-yr groups of rings taken from a 1-cm wide section across the tree. The wood was ground to pass through a 60 μm mesh sieve and solvent extracted first in a benzene-methanol mixture and then in acetone to remove lipids tars and resins. The remaining material was then dried in a vacuum oven for 3 d. Combustions were carried out in an evacuated nickel reaction vessel^{1,8}. $^{18}\text{O}/^{16}\text{O}$ ratios were measured on a 90° magnetic sector isotope ratio mass spectrometer and are expressed as a δ value ‰ with respect to standard mean ocean water (SMOW)⁹

$$(\delta^{18}\text{O}_x = [(^{18}\text{O}/^{16}\text{O})_x / (^{18}\text{O}/^{16}\text{O})_{\text{SMOW}} - 1] + 1,000\text{‰}).$$

Reproducibility of the whole wood analyses (and that of the cellulose analyses) was $\pm 0.2\text{‰}$, the same as for the mass spectrometer.

Cellulose was extracted from each of the 5-yr groups of rings using the method of Green¹⁰ which allowed the quantitative determination of α -cellulose, hemicellulose and lignin in each sample. (Care was taken to ensure standardisation of procedure for all samples.) Isotope ratios of solvent-extracted wood were found to be the same within experimental error as those of dry whole wood. Also, the isotopic compositions of hemicellulose and α -cellulose were found to be identical. This allowed the isotopic composition of the remainder (mostly lignin) to be calculated. Combination of the errors in the isotopic measurements of cellulose and whole wood, together with errors in measurements of percentage composition, yield an estimated error in the lignin isotopic composition of $\pm 0.6\text{‰}$. The results are given in Table 1.

The correlation coefficients of the isotope ratios of these components with seasonal temperature data were calculated. The results are shown in Table 2. For convenience the time period was divided into three-monthly groups such as January, February and March (Winter) and so on. The oxygen isotopic composition of cellulose is seen to have a significant correlation (95% confidence level) with mean seasonal temperatures throughout the year of growth. Correlation with mean annual temperature beginning with autumn previous to the calendar year of growth was found to be 0.94. The whole wood $^{18}\text{O}/^{16}\text{O}$ measurements show significant correlation only with mean temperatures for winter and spring of the calendar year (0.60) yielding a correlation coefficient with

Table 1 Oxygen isotopic compositions of cellulose, extracted whole wood and lignin

Sample	Cellulose $\delta^{18}\text{O}$ (‰)	Whole wood $\delta^{18}\text{O}$ (‰)	Lignin $\delta^{18}\text{O}$ (‰)	Lignin %	M.A.T.* (°C) (Sept–Aug)
1890–94	23.9	19.1	14.1	49.0	2.6
1895–99	23.4	19.6	15.4	47.4	2.3
1900–04	24.1	19.3	12.8	42.6	3.0
1905–09	24.7	19.9	15.2	50.5	3.1
1910–14	24.7	19.8	15.3	52.2	3.2
1915–19	24.1	19.6	15.1	49.8	2.7
1920–24	24.2	19.4	15.0	52.4	2.7
1925–29	23.6	19.1	14.5	49.3	2.3
1930–34	24.7	20.0	15.4	50.3	3.2
1935–39	22.9	18.3	13.2	47.2	2.1
1940–44	24.5	18.7	12.1	46.9	3.1
1945–49	23.3	17.7	11.2	46.2	2.5
1950–54	23.3	17.6	11.1	46.7	2.1
1955–59	24.5	19.4	13.8	47.6	3.1
1960–64	25.2	19.1	10.2	40.6	3.7
1965–68	24.0	18.7	12.5	46.0	2.4

Oxygen isotopic compositions ($\delta^{18}\text{O}$ against SMOW) of cellulose, extracted whole wood and lignin for 5-yr groups of tree rings, together with the lignin concentrations (%).

*5-yr mean annual temperatures calculated from September of the previous year to August of the final year.

mean annual temperature (September to August) of 0.55, significantly lower than that for cellulose. Correlation between $^{18}\text{O}/^{16}\text{O}$ ratios of lignin and mean annual temperatures (September to August) is essentially zero (-0.03). Also, there seems to be no relation between the amount of lignin present in the wood sample and seasonal temperature (Table 1). We conclude, therefore, that the lignin component of the wood carries no useful temperature information. The explanation for this may lie in the process of lignification of wood which takes place over a much longer period than that in which cellulose is produced and deposited in the tree ring¹¹. It is concluded, therefore, that the only temperature information contained in $^{18}\text{O}/^{16}\text{O}$ ratios of whole wood is due to that carried by the holocellulose component ($\approx 50\%$).

The overall good response of the cellulose isotopic composition to mean seasonal temperatures may be explained in terms of the integrating effect of the tree ring growth. Measurements of the isotopic composition of tree ring material may supply information on the temperatures prevailing during the production of sugars which are subsequently converted into cellulose. This may not coincide with the actual period of ring growth. The production of early wood in the tree ring is a rapid process which may use material stored in the tree as photosynthesis occurred late in the previous year and, to a lesser extent, throughout the winter. Late wood production, on the other hand, is likely to use material produced during the growth season of the tree. Thus, isotopic analysis of cellulose from tree rings may well provide an excellent integrator of mean annual temperature including a period before growth actually occurs.

In conclusion, for the tree species and climate zone being discussed, it seems that only cellulose $^{18}\text{O}/^{16}\text{O}$ ratios have a sufficiently good response with seasonal mean temperature to be used as a reliable indicator of past temperatures. Measurements of $^{18}\text{O}/^{16}\text{O}$ ratios of whole wood, although much faster to carry out, involve less work in extraction procedures and avoid possible contamination from chemical reagents, seem to yield much less reliable temperature information. Lignin, because of the difficulty

Table 2 Correlation coefficients of $^{18}\text{O}/^{16}\text{O}$ composition of wood fractions with mean seasonal and mean annual temperatures

	Autumn	Winter	Spring	Summer	Whole year
Cellulose	0.70*	0.80*	0.45*	0.42*	0.94*
Whole wood	0.30	0.52*	0.50*	-0.30	0.55*
Lignin	-0.10	0.10	0.12	-0.40*	-0.03

(Autumn = October, November and December and so on)

*Significant correlation at the 95% confidence level.

of carrying out direct $^{18}\text{O}/^{16}\text{O}$ measurements does not, at this time, present an alternative. We believe that this calibration procedure should be carried out for any species being considered for use as a climate indicator in a given climatic zone before the usefulness of cellulose or whole wood $^{18}\text{O}/^{16}\text{O}$ ratios can be evaluated.

This work was supported in part by grants from the NRC of Canada and the Atmospheric Environment Service of Canada.

J. GRAY*

P. THOMPSON

Department of Physics,
University of Alberta,
Edmonton, Alberta T6G 2J1,
Canada

Received 9 June; accepted 3 November 1977.

*Present address: Climatic Research Unit, School of Environmental Sciences, The University of East Anglia, Norwich, UK.

1. Gray, J. & Thompson, P. *Nature* **262**, 481–482 (1976).
2. Epstein, S. & Yapp, C. J. *Earth planet. Sci. Lett.* **30**, 253–261 (1976).
3. Yapp, C. J. & Epstein, S. *Earth planet. Sci. Lett.* **34**, 333–350 (1977).
4. Libby, L. M. & Pandolfi, L. J. *Nature* **266**, 415–417 (1977).
5. Wilson, A. T. & Grinstead, M. J. *Nature* **257**, 387–388 (1975).
6. Epstein, S. & Yapp, C. *Nature* **266**, 477–478 (1977).
7. Libby, L. M. & Pandolfi, L. J. *Nature* **266**, 478–479 (1977).
8. Thompson, P. & Gray, J. *Int. J. Rad. Isotopes* **28**, 411–415 (1977).
9. Craig, H. *Science* **133**, 1833–1834 (1961).
10. Green, J. W. in *Methods in Carbohydrate Chemistry* 3 (ed. Whistler, R. L.) (Academic, London, 1963).
11. Fritts, H. C. *Tree Rings and Climate* (Academic, London, 1976).

Chemical structure of humic substances

THE nature of soil humic acids and similar substances derivable from oxidised brown coal is of considerable fundamental and practical interest. It has now been found, using organic extractants, that solutions of soil humic substances, capable of NMR spectroscopic investigation, may be prepared. The spectra obtained suggest that polymers of a similar structure to oxidised polyethylene are important components of soils. Numerous other types of chemical structures are also evident from the NMR absorptions of soil organic matter solutions; the amounts and types present vary widely from soil to soil. It is suggested here that chemical fractionation of soil organic matter can be most easily followed by observation of the NMR spectra.

Previous studies of organic matter extracted from soils have mainly used degradative techniques (oxidative^{1,2}, pyrolysis–gas–liquid chromatography–mass spectrometry^{3,4}) or infrared spectroscopy^{5–10}. There is no general agreement on the chemical nature of soil organic matter. Interpretation of the infrared spectra of soil extracts has been hampered by the presence of complexed inorganic substances. Humic acids undoubtedly exist as complex organo–mineral–cation aggregates in nature¹¹, this probably contributing to their stability under soil conditions.

Attempts to separate the inorganic and organic part of soil humic substance molecules without undue disruption of the latter have not been particularly successful. The presence of inorganic contaminants undoubtedly obscures some of the fine details of the infrared spectra and profound band broadening effects may also be caused by the existence of 'acid salt' hydrogen bonding¹² in humic acids.

Extracts of humic materials obtained from soils by traditional aqueous alkaline solutions give ^1H -NMR spectra showing colloidal and solvent HO groups only. Progressive deuteration of the desiccated aqueous extracts leads to some resolution of the H–C groups in the NMR spectra but, owing to the difficulty of completely removing HO groups which cause band broadening by the formation of colloidal aggregates, the spectra obtained are poor quality. Also, the preferential solvation of O groups on the periphery of the macromolecules in aqueous systems apparently tends to remove the H–C structures from the solution phase, such hydrophobic groups occurring in a solid phase in the interior of

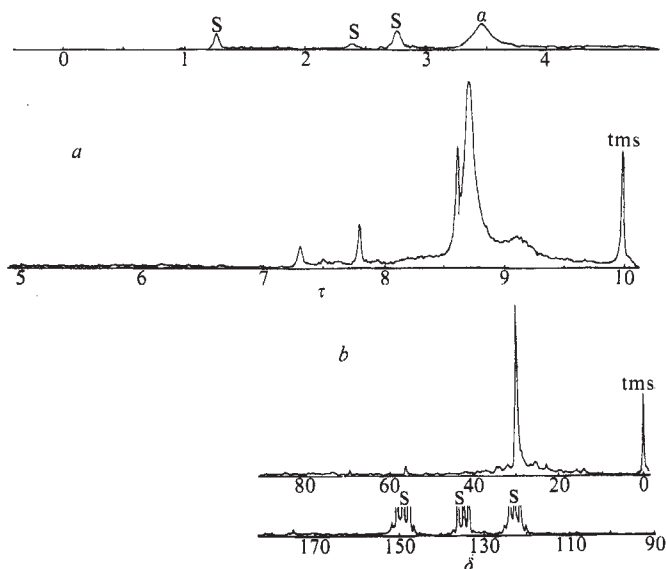
the macromolecules (which is NMR inactive under high resolution conditions).

^1H -NMR absorptions found in deuterio-dimethylsulphoxide or deuterio-pyridine solutions are much more distinct than the spectra (obtained in D_2O) of aqueous extracts and indicate the presence of $\text{CH}_3(\text{CH}_2)_n$, various environments of $\text{CH}_2(\text{CO})$, $\text{CH}_2\text{--NH}$ -, carbohydrate H–C–O, and usually to a minor extent, some aromatic groups. In general, spin–spin coupling is rarely detected in soil organic matter extracts, this being attributed to the presence of a large number of similar structures with overlapping peaks. Unsaturated groups are generally absent at NMR-sensitive concentrations. The $\text{CH}_3(\text{CH}_2)_n$ parts of the ^1H spectra resemble those of some crude oil GPC fractions¹³ but the ^{13}C Fourier transform spectra are apparently less similar to such materials.

Fractions of several soils were obtained by extracting successively with: (1) $(\text{CH}_3)_2\text{CO}$; (2) HCOOH ; (3) (4)* $(\text{CH}_3)_2\text{CO}$; (5)* 6M HCl ; (6) $(\text{CH}_3)_2\text{CO}$; (7) HCOOH ; (8) $(\text{CH}_3)_2\text{CO}$, by Soxhlet extraction or *cold extractions. This exhaustive extraction procedure removes essentially all the organic matter from the soil, and it can, therefore, be shown that polymethylene chains comprise up to ~30% of the total organic matter; however, fraction (1) may contain larger amounts of polymethylene chain components, as is demonstrated by the ^1H and ^{13}C NMR spectra shown in Fig. 1 (a and b respectively) obtained from a $(\text{CH}_3)_2\text{CO}$ Soxhlet extract of a podzol A horizon where 8% of the total organic matter was extracted. The ^{13}C NMR spectrum resembles that of polyethylene, the principal absorption present being attributed to $(\text{CH}_2)_n$.

The proportion of $(\text{CH}_2)_n\text{CH}_3$ present is best estimated from the ^1H NMR spectrum, Fig. 1a (the ^{13}C NMR spectrum was obtained under standard broad band ^1H decoupling conditions where the otherwise quantitative peak area proportionality with the numbers of resonating nuclei is inexact). This shows that 72% of the H–C groups of the humic fraction occurs in $(\text{CH}_2)_n\text{CH}_3$ structures, there being two superposed groups of peaks, one from (average) $n = 7$ at $\tau = 8.69$ ($w_{1/2}$, 6 Hz) and the other from $n \approx 20$ at $\tau = 8.7$ ($w_{1/2}$, ~60 Hz), the latter is suggested to be a fraction of semi-colloidal properties. This spectrum also indicates the presence of carbohydrate-like substances, $\tau = 5.1$ – 6.8 , ~6% of H–C and a small (<2%) amount of H–C (aromatic) at $\tau = 2.9$ and possibly also some (~3%) heteroaromatic groups at $\tau = 1.2$ – 2.0 . The remaining H–C at $\tau = 7.3$ – 8.6 is attributed to 'side chain' structures having H–C groups deshielded by O and N

Fig. 1 NMR Spectra of podzol humus, $(\text{CH}_3)_2\text{CO}$ Soxhlet extract Solvent: d-pyridine, tms internal standard; a, ^1H 60 MHz; b, ^{13}C , FT, 20 MHz spectra; a, τ value varies with dilution; s, solvent absorptions.



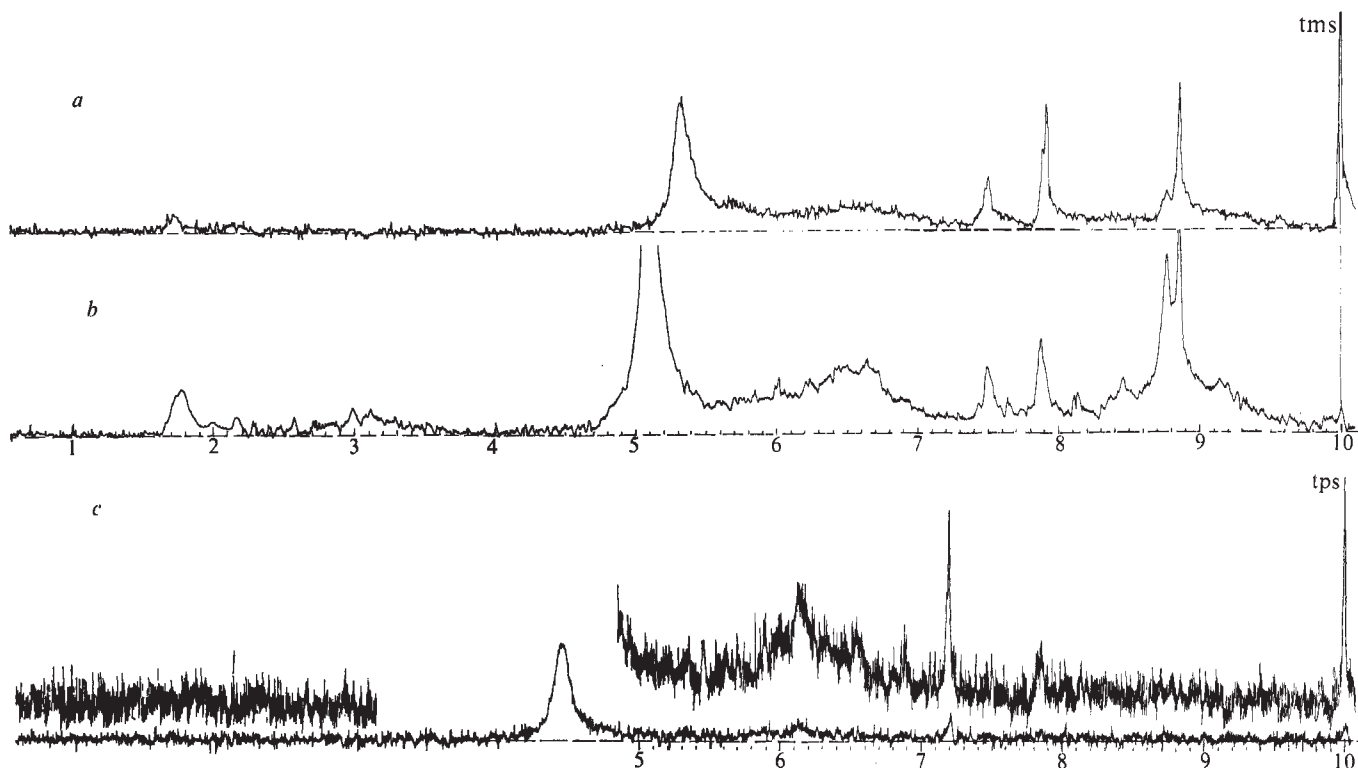


Fig. 2 NMR spectra of soil extracts, by HCOOH treatments. *a*, fraction (3) chernozem, in d-DMSO, tms internal standard; *b*, fraction (3) calcareous gley, in d-DMSO, tms internal standard; *c*, Zr^{4+} precipitated, deionised, HCOOH-DMSO (4:1) extract of Trawscoed E.H.F. 9-yr grazed ley; brown forest soil, in D_2O , tps [sodium, 3(trimethylsilyl)-l-propanesulphonate] internal standard.

atoms as well as by small (aliphatic) rings of C atoms. Some 1H NMR spectra of other organic matter fractions which are more typical of the main structural groups present in the firmer-bound fractions of soil organic matter, are shown in Fig. 2, where the amounts of $(CH_2)_nCH_3$ groups of the total H-C present are: spectrum *a*, 24%, spectrum *b*, 31% and spectrum *c*, 1-2%.

Our results agree with the relatively few literature reports of the use of NMR for humic acid structure determination¹⁴⁻²⁰, in particular the widespread occurrence of polymethylene-chain structures in humic acids is confirmed although the significance of this has not been previously noted. Spectra previously reported^{14,15,17} of a 'fulvic' acid extracted from a podzol B horizon^{14,15} and of humic acids extracted from lacustrine sediments¹⁷ are dominated by the absorptions of polymethylene chains and are similar to the spectrum shown in Fig. 1*a*.

The long-term stability of polymethylene structures, such as those present in high molecular weight synthetic polyethylenes, is well known and the persistence of such structures in the soil, in humic acid molecules and related substances, is likely to be due to the resistance of the higher molecular weight fractions of polymethylene-based polymers towards biodegradation.

Another form of non-carbohydrate-type of structure that occurs in the humic fractions of soil is apparently dominated by polyketide structures with various relatively short aliphatic chains joined to C=O groups. Such molecules, as constituents of humic materials, are also indicated from the published NMR spectra of organic matter obtained from river water²¹, where they probably occur as metal ion complexes. Part of the fraction (6) in the above scheme has been found to consist of such structures, the NMR spectra becoming evident upon the removal of complexed Fe(III) by treatment with $Na_4P_2O_7$ pellets in deuterio-dimethylsulphoxide medium.

A search of the literature for evidence in support of the NMR results from other techniques indicated that a microbial 'humic acid' prepared from *Aspergillus flavus* culture had an infrared spectrum suggesting the presence of polymethylene chains^{22,23}, indeed, a close similarity was indicated between this spectrum and

a published spectrum^{24,25} of oxidised branched polyethylene. This microbial humic acid had a very low ash content ($< 0.03\%$) and showed evidence of crystallinity. These are unusual features for substances which may be defined as humic acids; however, no soil humic acid has yet been prepared having such a low ash content and such a well resolved band structure in the infrared spectrum.

This work is supported financially by the ARC. I thank Professor J. Tinsley for stimulating discussion and for providing soil samples, also Mr M. Kibblewhite for providing soil extracts.

DAVID GRANT

Department of Soil Science,
University of Aberdeen,
Meston Walk,
Aberdeen, UK

Received 3 August; accepted 28 October 1977.

- Ogner, G. & Gjessing, E. T. *Geoderma* **14**, 139-145 (1975).
- Schnitzer, M. & Neyroud, J. A. *Soil Biol. Biochem.* **7**, 365-371 (1975).
- Bracewell, J. M. & Robertson, G. W. *J. Soil Sci.* **24**, 421-428 (1973).
- Nagar, B. R. *Nature* **199**, 1213-1214 (1963).
- Schnitzer, M. & Skinner, S. I. M. *Isotop. Radiat. Soil Org. Matter Stud. Proc. Symp. 2nd Int.* **41-55** (1968).
- Otsuki, A. & Hanya, T. *Nature* **212**, 1462-1463 (1966).
- Boldyrev, A. I., Malyushenko, B. T. & Polyakov, A. A. *Pochvovedenie* **81-88** (1969) *Soviet Soil Sci.* **222-228** (1969).
- Thompson, S. O. & Chesters, G. J. *Soil Sci.* **21**, 265-272 (1970).
- Shurukhina, S. I., Shurukhin, V. V. & Tarlakov, Yu. P. *Pochvovedenie* **146-149** (1973) *Soviet Soil Sci.* **240-243** (1973).
- Bailey, J. R. *Plant and Soil* **40**, 285-302 (1974).
- Bremner, J. M. *J. Soil Sci.* **5**, 214-232 (1954).
- Shrivastava, H. N. & Speakman, J. C. *J. chem. Soc.* **1151-1163** (1961).
- Coleman, H. J., Hirsch, D. F. & Dooley, J. E. *Analyt. Chem.* **41**, 800-804 (1969).
- Barton, D. H. R. & Schnitzer, M. *Nature* **198**, 417-418 (1963).
- Schnitzer, M. & Skinner, S. I. M. *Isotop. Radiat. Soil Org. Matter Stud. Proc. Symp. 2nd Int.* **41-55** (1968).
- Oka, H., Sasaki, M., Itoh, M. & Suzuki, A. *Neuro Kyokai-shi* **48**, 295-302 (1969).
- Ishiwatari, R. *Chem. Geol.* **12**, 113-126 (1973).
- Lüdemann, H.-D., Lentz, H. & Ziehm, W. *Erdöl und Kohle-Petrochemie-Brennstoff Chemie* **26**, 506-509 (1973).
- Gonzalez, Vila, F. J., Lentz, H. & Lüdemann, H. D. *Biochem. biophys. Res. Commun.* **72**, 1063-1076 (1976).
- Sciavocelli, O., Senesi, N., Solinas, V. & Testini, C. *Soil Biol. Biochem.* **9**, 287-293 (1977).
- Reuter, J. H. & Perdue, E. M. *U.S. natn. Tech. Inform. Serv. Rep.* PB 210 714 (1972).
- Visser, S. A. W. *Afr. J. Biol. appl. Chem.* **13**, 3-13 (1970).
- Visser, S. A. & Mendel, H. *Soil Biol. Biochem.* **3**, 259-265 (1971).
- Raff, R. A. V. *Ethylene and its Industrial Derivatives* (ed. Miller, S. A.) 377 (Benn, London, 1969).
- Brandrup, J. & Immergut, E. H. *Polymer Handbook* **7**, 48-51 (Interscience, New York, 1966).

Origin of stanols in young lacustrine sediments

STEROIDAL skeletons have been widely used as markers of the biological origin of organic material in ancient sediments¹⁻⁷ and petroleum⁸. Nevertheless, the origin and fate of sterols (stanols and stenols) in recent sediments are poorly understood. Here the significant contribution of organism-derived stanols (saturated sterols) to lacustrine sediments is reported and the geochemical significance discussed. The presence of stanols in recent and ancient sediments, together with unaltered stenols (unsaturated sterols) commonly found in algae, has been reported⁹⁻¹². In view of the rare abundance of stanols in living organisms, this occurrence has been used as evidence that partial reduction of naturally occurring stenols had taken place over geological time. Stanols have also been identified in contemporary lacustrine sediments¹³⁻¹⁷. Based on the conversion of ¹⁴C-cholesterol into ¹⁴C-cholestanol under *in situ* incubation, Gaskell and Eglington^{17,18} proposed that such stanols in young sediments originate from stenols by microbiological reduction after deposition.

More recently, the same stanols found in contemporary lacustrine sediments were identified in phytoplankton, zooplankton^{15,19,20} and various higher plants²¹, although in very small amounts (two or three orders of magnitude less than the total amount of sterol). In various surface soils, however, (0-3 cm in depth) and in the oxidising layer (0-1 cm in depth) of Suwa surface sediments, significant quantities of stanols were found²¹. Of the total sterol in the sediment samples, ~ 20% was composed of stanols. From these findings and from the difference in the degradation rates for stanols and stenols observed in incubation experiments, Nishimura and Koyama¹⁹ concluded that stanols from organisms were concentrated, particularly under oxidative conditions, by the preferential degradation of stenols.

These results suggested that stanols in various sediments were derived not only from the stanol hydrogenation during sedimentation, but also directly from various living organisms. It is difficult, however, to estimate precisely the respective contribution in lacustrine sediments from the organisms and from the hydrogenation. The examination of a contemporary sediment in which virtually no stanol hydrogenation takes place would provide information on the contribution of organism-derived stanols to contemporary lacustrine sediments. The sediment is from Lake Shirakome-ike, Nagano, Japan (maximum depth, 8.6 m; area, 0.1 km²) which is an oligotrophic lake containing humic substances located about 2,100 m above sea level in the Yatsugatake mountain chain. Most of the sedimentary organic material may, therefore, be derived from terrigenous sources. The bottom water is oxygenated all year round. The sediment temperature ranges from 2 to 7 °C throughout the year.

Hayashi²² investigated the microbial activity at four levels of the surface sediments (0-12 cm in depth). He found that the population (10⁴-10⁵ per g dry mud) of heterotrophic bacteria in the sediment layers was two orders of magnitude less than in Lake Suwa sediments in which stanol hydrogenation took place¹⁹, and that a greater proportion of the bacteria were spore-former types. On the other hand, the concentration of dissolved oxygen in the water during the stagnation period (November-July) virtually did not decrease with depth²³ and the consumption rate (9 mg O₂ m⁻² d⁻¹) of O₂ in the surface sediments was very slow relative to other lacustrine sediments²². These findings indicate that the microbiological activity in the surface sediments is extremely low.

Sediment samples were taken from the same core used by Hayashi²². The sample was collected from the deepest part of the lake in summer and was divided into sections at 3 cm intervals. A plankton sample was also collected with a plankton net in summer (the bloom season). Most of the plankton species were zooplankton, with the main species *Acanthodiptomus* sp. (~ 60%) and *Daphnia* sp. (~ 20%)²⁴. All samples were frozen immediately and remained frozen until analysed.

The lipid extraction and the procedure for sterol analysis were as described by Nishimura and Koyama¹⁹. Structural identification of the sterols, following derivatisation to trimethylsilyl (TMS) ethers, was based on gas-liquid chromatography with co-injection of the authentic sterols on two types of columns and comparison of the mass spectra with those of authentic sterol TMS ethers^{13,25}. The stereochemical assignment of the alkyl group at C-24 in C₂₈ and C₂₉-sterol was not determined.

No interconversion of stenols to stanols was detected in control experiments employing the same procedures. The concentrations of total organic carbon, nitrogen and lipid carbon were obtained on a CHN analyser (Yanagimoto Model MT-1S).

Sterols (stanols and stenols) characteristically were distributed in some plankton species abundant in fresh-water lake and terrigenous sources, respectively; cholesterol and 5 α -cholestan-3 β -ol (C₂₇-sterols) are predominant in the plankton examined¹⁹ and 24-ethyl-cholesterol and 24-ethyl-5 α -cholestan-3 β -ol (C₂₉-sterols) in various plants and soils²¹. Therefore, the sterols in lacustrine sediments provide a useful indicator for estimating the autochthonous vs terrestrial contribution to the sedimentary organic matter. Table 1 summarises the sterol constituents identified in Shirakoma sediments and in zooplankton abundant in the lake. The major components in the sediments were 24-ethylcholesterol and 24-ethyl-5 α -cholestan-3 β -ol, while in the plankton cholesterol and 5 α -cholestan-3 β -ol predominated. Moreover, the carbon number (C₂₇, C₂₈ and C₂₉) distribution of sterols in the sediments was very similar to those of *Larix* sp., abundant in the forest around the lake and in soil samples²¹. These facts indicate that most of the organic matter in the surface sediments was terrestrial in origin.

Total organic carbon, nitrogen, C/N ratio and lipid carbon in the surface sediments (0-20 cm in depth) are summarised in

Table 1 The major sterol components of the sediments and plankton from Lake-Shirakoma-ike

Sterol	0-3		Core sample (depth in cm)				12-20		Plankton
	(μ g)*	(%)†	(μ g)	(%)	(μ g)	(%)	(μ g)	(%)	
Cholesterol	8.4	12	8.0	11	5.3	13	6.4	9	95
24-methylcholest-5,22-diene-3 β -ol	trace	—	6.6	9	trace	—	2.1	3	ND‡
24-methylcholesterol	8.4	12	10.3	14	4.1	10	8.6	12	5
24-ethylcholest-5,22-diene-3 β -ol	7.7	11	8.8	12	4.1	10	7.1	10	trace
24-ethylcholesterol	45.8	65	39.7	54	27.6	67	47.3	66	trace
Stanol									
5 α -cholestan-3 β -ol	6.9	12	4.5	13	3.2	8	4.2	9	76
24-methyl-5 α -cholestan-3 β -ol	5.4	9	3.8	11	4.1	10	4.6	10	8
24-ethyl-5 α -cholestan-3 β -ol	43.6	77	26.6	76	33.7	82	37.8	81	16
Total stanol	70.3		73.9		41.1		71.5		
Total stanol	55.9		34.9		41.0		46.6		
Total stanol/total sterol	0.44		0.32		0.50		0.39		0.006

* μ g/g dry sediment.

†The percentage of total stanol and total stanol.

‡ND, not detected.

Table 2 The vertical distribution of total organic carbon (T.O.C.), nitrogen (T.O.N.), C/N ratio and lipid carbon (Lipid C) in Shirakoma sediments

Depth (cm)	T.O.C.	T.O.N (mg C(or N) per g dry sediment)	Lipid C	C/N
0-3	242	16	8.0	15
3-6	240	14	8.1	17
9-12	258	16	8.1	16
12-20	265	16	8.1	16

Table 2. All of the organic parameters were fairly constant irrespective of the sediment depth. In addition, the ratio of amino acid-, amino sugar-, insoluble- and NH_4^+ -N to total nitrogen was constant throughout the sediment depth²². These facts strongly indicate that the microbiological activity in the sediments is greatly suppressed. This suppression is presumably due to the depletion of metabolisable organic compounds in the sediments²⁶ along with the low temperature (2–7 °C) of the sediment, because most of the organic matter is terrigenous material, composed mainly of diagenetically formed, secondary reaction products, like humic substances and kerogen²⁷. Stenol hydrogenation in sediments, however, is a process effected by bacterial activity^{17,19}. Thus, the contribution of stanols by way of the stenol hydrogenation process to the sediments, if any, may be extremely small.

Large quantities of stanols were found in all the sediment levels examined (Table 1). Particularly at the surface level (0–3 cm in depth), 44% of total sterol was composed of stanols. The relative abundance of stanols in the remaining levels ranged from 32 to 50%. The range was larger than that (20–30%) found in Suwa surface sediments (0–10 cm in depth) where stenol hydrogenation took place¹⁹. The high relative amounts of stanol in Shirakoma sediments presumably resulted from the concentration of stanol derived from organisms by the preferential degradation of stanols under oxidative conditions, until incorporation into the bottom sediments.

The plankton mixture from the lake yielded a very small amount of stanols (two orders of magnitude less than the amount of stenols) (Table 1). The major stanol was 5 α -cholestan-3 β -ol. In comparison, ~80% of total stanol in the sediments was 24-ethyl-5 α -cholestan-3 β -ol (Table 1). This stanol is a major component in terrigenous-derived material (land plants and soils)²¹. This indicates that stanols derived from organisms on land may contribute significantly to stanols found in lacustrine sediments, which contain major proportions of terrigenous organic materials. Therefore, the relative abundances of C₂₇-sterol (plankton origin) and C₂₉-sterol (terrestrial origin) in young sediments may provide a strong clue to the origin of stanols (from organisms and/or from microbiological hydrogenation of stenols).

Gaskell and Eglinton¹⁷ reported that stanols in Rostherne Mere sediment (0–30 cm in depth) were derived from stenol hydrogenation during burial. But the striking predominance of C₂₉-sterols (24-ethylcholesterol + 24-ethyl-5 α -cholestan-3 β -ol) in both 7–18 cm and 18–30 cm levels of Rostherne sediment indicates that most organic materials were derived from terrigenous sources. This, coupled with the low temperature of Rostherne sediment (6–10 °C), indicates that most of the stanols in both 7–18 cm and 18–30 cm levels might originate directly from organisms on land by the preferential degradation of stanols during transportation to the bottom sediments. This may also be the case with the stanols in the 24–26 cm and 30–36 cm levels of Suwa sediments²¹.

Thus, the possible significant contribution of organism-derived stanols to sediments is not necessarily evidence for the operation of microbiological and/or chemical reduction of stanols during preservation. It is hoped that the ability to discriminate between stanols derived from the hydrogenation process and from organisms will provide information essential for understanding the origin and fate of steroid compounds in sediments.

In view of the scarcity of stanols in living organisms (land plants and various plankton species)^{19,21}, the relative abundance

of stanols from organisms in early sedimentation may be a useful indicator of the operation of diagenetic processes under oxidative conditions. For example, the ratio of stanol to sterol (stenol + stanol) in Shirakoma sediments, various soils and Suwa surface sediments may provide information on the degree of diagenetic alteration undergone by the organic materials in the same sediments under oxidative conditions.

I thank Professor Hanya and Dr Ishiwatari of Tokyo Metropolitan University for providing GLC-MS facilities, also Professor T. Koyama of Nagoya University, Water Research Institution, for his helpful comments.

MITSUGU NISHIMURA

Water Research Institute,
Nagoya University,
Chikusa-ku Nagoya,
Japan

Received 19 August; accepted 28 October 1977.

- Burlingame, A. L., Huang, P., Belsky, T. & Calvin, M. *Proc. natn. Acad. Sci. U.S.A.* **54**, 1406–1412 (1965).
- Anderson, P. C., Gardner, P. M., Whitehead, E. V., Anders, D. E. & Robinson, W. E. *Geochim. cosmochim. Acta* **33**, 1304–1306 (1969).
- Kimble, B. J. *et al. Geochim. cosmochim. Acta* **38**, 1165–1181 (1974).
- Henderson, W., Wollrab, V. & Eglinton, G. in *Advances in Organic Geochemistry* (eds Schenck, P. A. & Havenaar, J.) 181–208 (Pergamon, Oxford, 1968).
- Anders, D. E. & Robinson, W. E. *Geochim. cosmochim. Acta* **35**, 661–678 (1971).
- Gallegos, E. J. *Anal. Chem.* **47**, 1523–1528 (1975).
- Mulheirn, L. J. & Ryback, G. *Nature* **256**, 301–302 (1975).
- Hills, I. R., Smith, G. W. & Whitehead, E. V. *J. Inst. Petrol.* **56**, 127–137 (1970).
- Attaway, D. & Parker, P. L. *Science* **169**, 674–675 (1970).
- Mattern, G., Albrecht, P. & Ourisson, G. *Commun.* 1570–1571 (1970).
- Steel, G. & Henderson, W. *Nature* **238**, 148–150 (1972).
- Ogura, K. & Hanya, T. *Proc. Japan. Acad.* **49**, 201–204 (1973).
- Henderson, W., Reed, W. E. & Steel, G. in *Advances in Organic Chemistry* (eds von Gaertner, H. R. & Wehner, H.) 335–352 (Pergamon, Oxford, 1971).
- Gaskell, S. J. & Eglinton, G. in *Advances in Organic Geochemistry* (eds Tissot, B. & Biennier, F.) 963–976 (Editions Technip, Paris, 1974).
- Nishimura, M. & Koyama, T. *Chem. Geol.* **17**, 229–239 (1976).
- Lee, C., Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **41**, 985–992 (1977).
- Gaskell, S. J. & Eglinton, G. *Geochim. cosmochim. Acta* **40**, 1221–1228 (1976).
- Gaskell, S. J. & Eglinton, G. *Nature* **254**, 209–211 (1975).
- Nishimura, M. & Koyama, T. *Geochim. cosmochim. Acta* **41**, 379–385 (1977).
- Isabell, C., Masuo, M. & Ikekawa, N. *Phytochemistry* **15**, 723–725 (1976).
- Nishimura, M. *Geochim. cosmochim. Acta* (in the press).
- Hayashi, H. *Jap. Soc. Ecology Abstr. A. Meet.* **23**, 23 (1976).
- Kurasawa, H. & Aoyama, K. *Miscellaneous Rep. Res. Inst. Natur. Resource* **63**, 9–16 (1964).
- Okino, T. *Jap. Soc. Ecology Abstr. A. Meet.* **23**, 22 (1976).
- Brooks, C. J., Henderson, W. & Steel, G. *Biochim. biophys. Acta* **296**, 431–445 (1973).
- Sorokin, Y. I. *Microbiologia* **3**, 402–413 (1962).
- Welte, D. H. in *Advances in Organic Chemistry* (eds Eglinton, G. & Murphy, M. T. J.) 261–264 (Springer-Verlag, New York, 1969).

Flandrian sealevel changes in the Thames Estuary and the implications for land subsidence in England and Wales

CATASTROPHIC flooding occurred in the Lower Thames and lowland coastal areas of eastern England in 1953. Large-scale investment in port, freight, factory facilities and connected urban development, has centred in these littoral and coastal zones. The effects of changing sealevels, such as seen in 1953, could, therefore, cause even greater economic and human loss in the future, unless government expenditure on flood alleviation schemes, as exemplified by the Thames barrage project, is increased and a coordinated flood protection policy established. Information on the rate of changing relative sealevel in south-east England is thus of great value. (A description is given here of a stratigraphic study upon the interleaved Flandrian biogenic and inorganic deposits of the Lower Thames estuary, carried out between central London and the Isle of Grain (Fig. 1).) From this, the heights of relative sealevel movements were determined and plotted against time to show the rate of relative sealevel change and subsidence trends for the Thames and southern England. The vegetational and environmental history was deduced from pollen, diatom and other micro-fossil analyses, with radiocarbon dating applied to establish an objective chronology.

The Flandrian sequences are developed on a fluviially-dissected late Devonian gravel surface, which falls progressively in height from west to east. The intercalated alluvial deposits continue this west-east dip, increasing in degree from

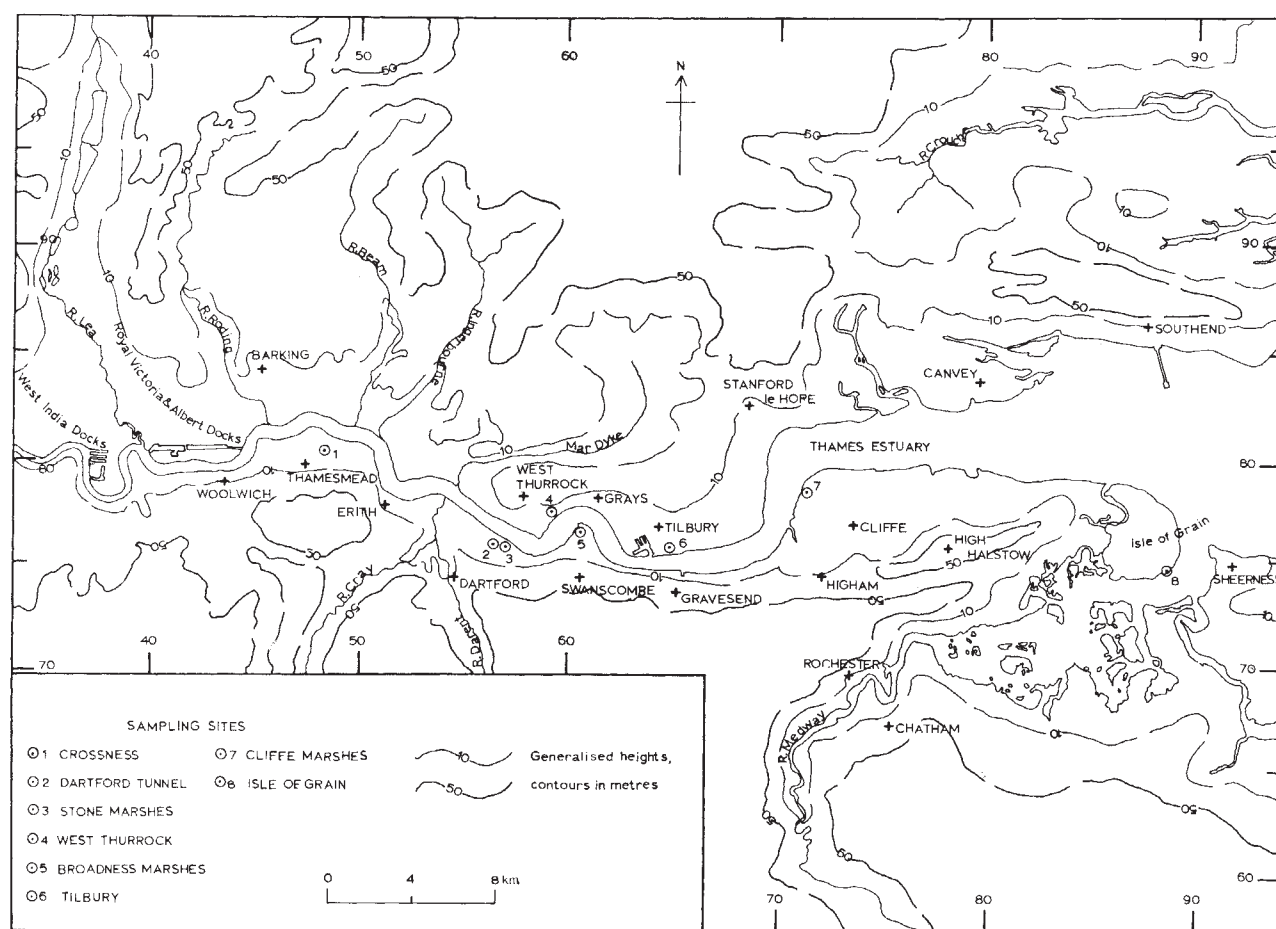


Fig. 1 Sketch map of the Lower Thames area.

Broadness Marsh eastwards. The inorganic fraction is composed of fine blue grey muds $<60\ \mu\text{m}$ in diameter, with silt and clay-sized particles dominant. From these phases of inorganic deposition five marine transgressions are recognised (see Table 1).

A complete profile of the diatom assemblages from Tilbury, in conjunction with other diatom and malacological studies, shows the importance of brackish water and tidal influences in the estuary from the outset in Thames I. The full marine effect was established soon after in the sequence, as characterised by the diatoms *Melosira sulcata*, *M. westii*, *Cymatosira belgica*, *Coscinodiscus excentricus* and *Raphoneis* spp. Towards the top of the deposits a fresh/brackish water influence becomes dominant. This shows a lessening of the marine effect nearer the present day, and is consistent with the accompanying decline in the rate of relative sealevel rise (Fig. 2).

The biogenic layers, formed by wood and monocotyledonous peats with gyttjas, show distinct internal changes in composition along an east-west transect. At the eastern end, *Phragmites* and saltmarsh peats dominate the deposits, changing upstream to form freshwater oak-alder fen wood peat. West of Broadness Marsh, all the sites showed seral vegetational development over the contact zones in the biogenic layers, changing from wood fen peat to *Phragmites* and then saltmarsh peat before the close and also before the onset of a marine transgression. This vegetational pattern supports the diatom evidence of an early and increasing salinity influence downstream and the rapid upstream progression of the tidal limit towards central London.

Pollen and macro-fossil analyses show the importance of the local influences of saltmarsh and fen environments on the vegetational history of the area. Arboreal pollen of *Alnus*, *Quercus*, *Corylus* type and *Tilia* occur in high frequencies

throughout. Following the recognition of the *Ulmus* decline at the Thames site at about 5,000 yr BP, non-arboreal pollen becomes dominant in the area's pollen rain, reflecting both increasing anthropogenic activity and rising sealevel. In a regional context, the rational limit of *Alnus* pollen¹ has been placed between $8,510 \pm 110$ and $8,170 \pm 100$ yr BP, with high *Alnus* frequencies occurring in a late Boreal Vlc pollen context². This dating is the earliest established at present for the rise of alder in south-east, central southern England and East Anglia. As such, it indicates the importance of wet valley conditions and related physiographic factors on the growth and expansion of this taxon in the Flandrian. From the biogenic deposits five regression phases, Tilbury I-V, have been recognised throughout the area, with Tilbury forming the type site.

From 19 radiocarbon-dated index points, taken from the regression and transgression contacts of the interleaved deposits, relative sea-level curves have been drawn for the area (Fig. 2). These contacts show the contemporary positions of MHWST, as determined by the biostratigraphic analyses. Compaction and consolidation corrections have not been used. Use of such corrections assumes a detailed geotechnical knowledge of the *in situ* behaviour of interleaved inorganic and biogenic deposits, which as yet has not been demonstrated^{3,4}. Further, corrections from individual cores cannot be used by themselves to represent regional changes of level satisfactorily, because of the great local variability of the sediments. From Fig. 2 some general conclusions about the movement of sealevel can be drawn for the Lower Thames:

(1) 8,500/300–7,000 yr BP showed a rapid rise of sealevel from $-25.5\ \text{m}$ to $-8.9\ \text{m}$ OD (ordnance datum). Peat growth occurred on the basal Devonian sand and gravel surface, dependent on the rising freshwater table. No regression phases were recorded during this time. The rate of submerg-

ence was approximately 1.3 cm yr^{-1} , compared with 1.2 cm yr^{-1} for the Netherlands⁵ and 1.5 cm yr^{-1} for north-west England⁶.

(2) Sealevel fell for about 300 yr between 7,000 and 6,700 yr BP, with formation of alder wood peat.

(3) During Thames II sealevel rose between 6,600 and 5,500 yr BP from -10.1 m to -5.0 m OD . This forms the most extensive transgression recorded in the sequence, although the relative sealevel rise has fallen to 0.5 cm yr^{-1} compared to 0.36 cm yr^{-1} for the Netherlands.⁵

The 'best fit' line for the behaviour of sealevel, expressing the positive and negative movements represented by the intercalated clays and peats, is an oscillating curve (Fig. 2). A mean line for the index points, forming the relative sealevel curve 4, is also produced to show the overall trend of a steadily rising sealevel with time. These curves show a strong similarity in the form and rate of relative sealevel change with those of north-west Europe⁷. The timing and amplitude of movement in the Thames disagrees with the sealevel curve produced for the Essex coast⁸. This is probably because of the differing

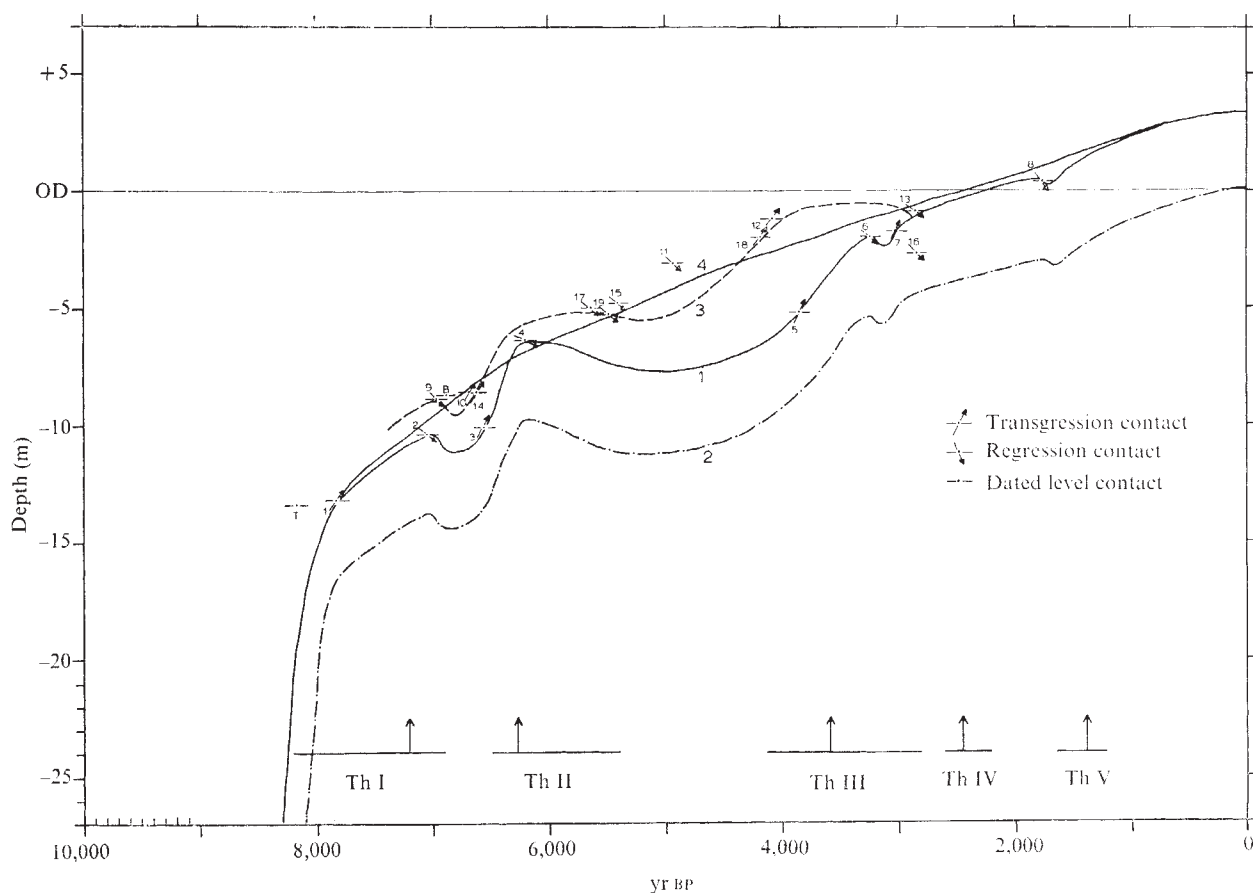


Fig. 2 Relative sea-level curves for the Thames Estuary. 1, Relative movement of mean high water spring tides (MHWST) at Tilbury. 2, Relative movement of mean tide level (MTL), derived from the difference between MHWST and MTL of the sample contact points. 3, Relative movement of MHWST from Crossness, Stone Marsh, Dartford Tunnel and Broadness. 4, Curve representing the mean line for the sampled contact points. Th I-V are transgression sequences recognised in the Thames estuary. Index points are as follows: 1-8, Tilbury (T); 9-13, Stone Marsh; 14-16, Broadness Marsh (B); 17-18, Crossness; 19, Dartford Tunnel. IG, Isle of Grain.

(4) Between 5,500/5,000 and 4,000 yr BP a major regression, Tilbury III, took place with formation of thick monocotyledonous peat, becoming wood fen peats upstream. Environmental conditions in the estuary would not have been sensitive enough to record more minor fluctuations in sealevel, shown for this time in the open coastal sediments of north-west England⁶ (Fig. 3). (5) Thames III shows a rapid rise in sealevel between 4,000 and 3,000 yr BP to levels between -1.4 m and -2.5 m OD . A minor regression phase Tilbury IV is recorded in the form of a thin silty peat. The timing and height of this event varies in the estuary and may result partly from different rates of sedimentation and relative subsidence in the estuary.

(6) Following this regression, sealevel rises above present ordnance datum (Newlyn), reaching $+0.4 \text{ m OD} \sim 1,750 \text{ yr BP}$ at Tilbury. At this level, a thin non-persistent silty peat records a further regression termed Tilbury V.

(7) During Thames V, over the last 1,000 yr, sealevel for MHWST continued to rise rapidly, possibly reflecting increased embanking and building in the estuary.

environmental and sedimentological histories of the two areas, in addition to the wide variety of data sources used by Green-smith and Tucker for this area. Despite the local and regional inaccuracies implicit in sealevel data^{9,10}, comparison of Flandrian relative sealevel curves from similar area-based studies in England and Wales has allowed trends in land subsidence to be drawn. Within the Thames, possible differential down-warping of about 1.5 m has been identified between Crossness and Tilbury for the Flandrian, shown by the differential between curves 1 and 3 (Fig. 2). Changes upstream in

Table 1 Flandrian transgression sequences in the Lower Thames estuary

Thames V	..	..	..	..	..	$\sim 1,750 \text{ yr BP}$
Thames IV	..	..	..	..	..	2,600- / yr BP
Thames III	..	..	..	..	..	3,850-2,850 yr BP
Thames II	..	..	..	..	..	6,575-5,410 yr BP
Thames I	..	..	..	..	..	8,200-6,970 yr BP

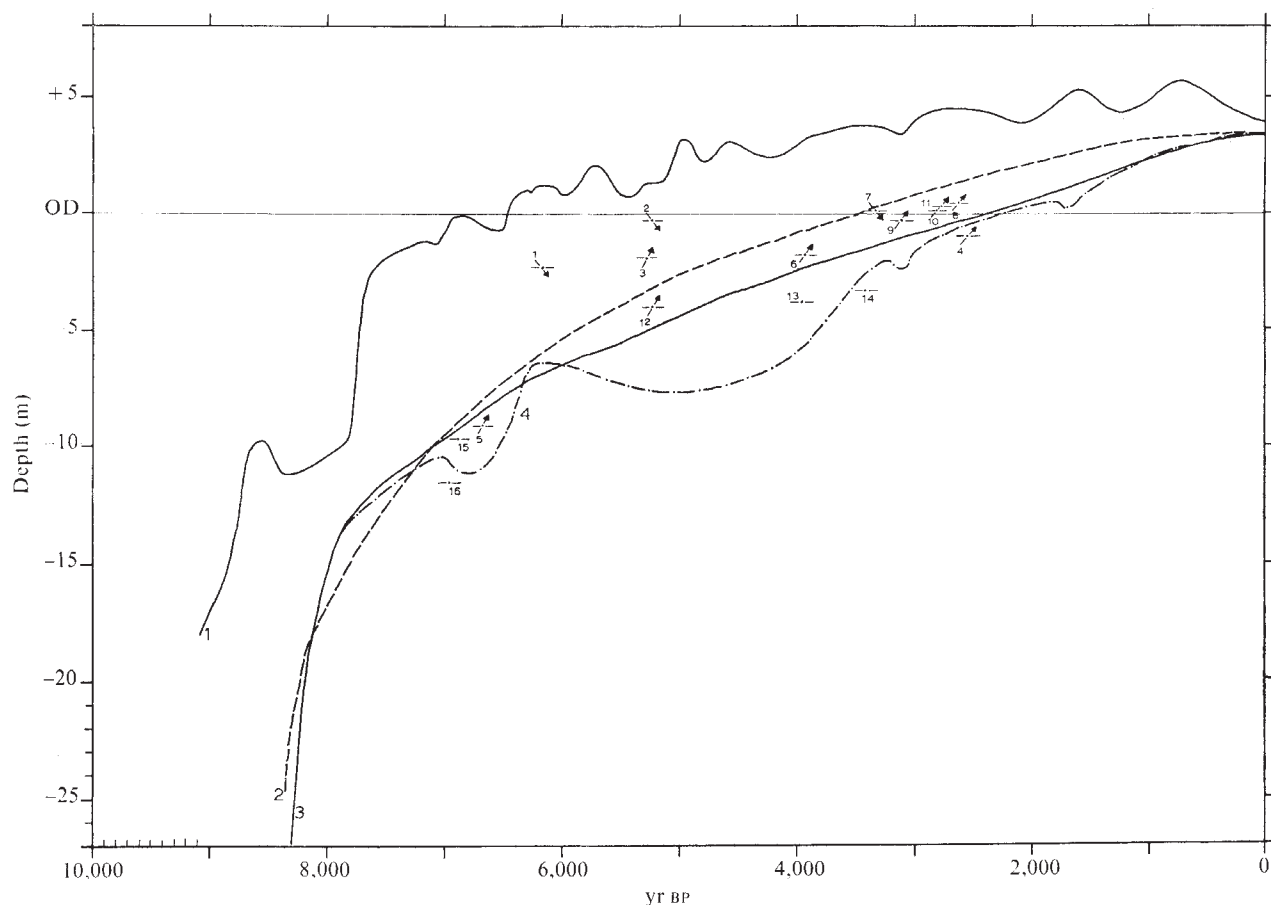


Fig. 3 Relative sealevel curves indicating subsidence trends in England and Wales. Transgression (upward arrow) and regression (downward arrow) contacts from intercalated peat and clay deposits in north-east and eastern England¹⁵. Relative sealevel curve 1 from north-west England⁶; curve 2 from south-west England (R.J.D. in preparation); curve 3 from the Lower Thames estuary (see Fig. 2); curve 4 represents the movement of MHWST at Tilbury, Lower Thames estuary (Fig. 2 and ref. 16).

the tidal amplitude in the estuary, however, may form an important component here. The regional trends of relative west-to-east and north-to-south down-warping in England¹¹ are supported (Fig. 3). By comparison, the amount of relative land subsidence for south-east England relative to the south-west, formerly given as 6.1 m since ~ 6,500 yr BP¹², is not confirmed. This figure was based on inadequate field data and a lack of understanding of the inequalities in cross-continental correlations of sealevel movements—the changes in geoidal configuration, tectonic stability and tidal amplitude. Comparison of the curves derived from biostratigraphic and ¹⁴C analyses of the transgression and regression contacts of intercalated deposits in these two areas, shows a consistent trend of down-warping of the south-east relative to the south-west. The value for this relative subsidence may be placed at 2–3 m for the Flandrian¹⁶. But, rate of down-warping are seen to vary with many local environmental parameters, for example, compaction and consolidation ratios, tidal regimes¹³ and changes in the height of the regional sea surface^{10,14}, making generalisation of subsidence values tenuous.

R. J. N. DEVOY

*Sub-department of Quaternary Research,
Botany School, Downing Street,
Cambridge, UK*

Received 16 June; accepted 1 October 1977.

1. Smith, A. G. & Pilcher, J. R. *New Phytol.* **72**, 903–914 (1973).
2. Godwin, H. *The History of the British Flora*. (Cambridge University Press, Cambridge, 1975).

3. Skempton, A. W. *Q. Jl geol. Soc. Lond.* **125**, 373–412 (1970).
4. Marsland, A. Q. *J. Engng. Geol.* **10**, 1–26 (1977).
5. Jelgersma, S. *Meded. Geol. Sticht.* Ser. C **6**, 1–100 (1961).
6. Tooley, M. J. *Geol. J.* **11**, 137–152 (1976).
7. Morner, N.-A. *Palaeogeogr. Palaeoclimatol. Palaesecol.* **19**, 63–85 (1976).
8. Greensmith, J. T. & Tucker, E. V. *Geol. en Mijnb.* **52**, 193–202 (1973).
9. Tooley, M. J. *Geogr. J.* **140**, 18–42 (1974).
10. Morner, N.-A. *J. Geol.* **84**, 123–151 (1976).
11. Dunham, K. C. *Phil. Trans. R. Soc. Lond. A* **272**, 81–86 (1972).
12. Churchill, D. M. *Quaternaria* **7**, 239–249 (1965); *Proc. prehist. Soc.* **5**, 74–84 (1965).
13. Bowen, A. J. *Phil. Trans. R. Soc. Lond. A* **272**, 187–199 (1972).
14. Rossiter, J. R. *Geophys. J. R. astr. Soc.* **12**, 259–299 (1967).
15. Gaunt, G. D. & Tooley, M. J. *Bull. Inst. geol. Sci.* **48**, 25–41 (1974).
16. Devoy, R. J. thesis, Univ. Cambridge.

Are spreading centres perpendicular to their transform faults?

A COMMON assumption in seafloor spreading is that mid-ocean ridge crests are aligned perpendicular to their transform faults and, hence, to their spreading directions. There are some well known exceptions to this rule, for example, the Reykjanes Ridge. Vogt *et al.*¹ suggested that spreading systems may take one of two configurations: either a transform faultless, oblique configuration or a perpendicular one. He then assigned the Reykjanes and certain older anomaly sets to the first category and the rest, the segmented, faulted ridges to the latter. We agree with this bimodal separation of ridge types, and here we discuss only the latter, the transform-faulted 'perpendicular' group. We examined all available detailed data from this group, and wherever we could

find a fine scale map which included both transform fault and spreading centre we measured their trends. Of eight segmented, slow-spreading centres (half-rate less than 3 cm yr^{-1}) we did not find a case which was, in fact, perpendicular. All were $6\text{--}38^\circ$ oblique, and all were oblique in the sense which shortens the connecting transform faults, that is, the configurations in Fig. 1a as opposed to those shown in Fig. 1b. Fast- and some intermediate-rate spreading centres, on the other hand, seem to be perpendicular within the errors of measurements. These results are particularly interesting for the constraints that they place upon models of spreading centres in which the ridge crest-transform fault angle is used as a measure of the relative amounts of energy dissipated by these two features as motion occurs across them.

While working on the masses of data recently collected in the famous area, we became convinced that the spreading centres are not perpendicular to the transform faults here. They are aligned 17° oblique to the spreading normal direction and their obliqueness is such that the transform faults are shortened (C, R in Fig. 1). Furthermore, this obliqueness has been quite stable through time. Before about 5 Myr ago, the spreading direction seems to have been about $N 105^\circ E$ while the spreading centre trend was about $N 35\text{--}45^\circ E$, or about $20\text{--}30^\circ$ oblique to the spreading normal. Between 5 and 3 Myr ago, the direction changed to E-W, causing the spreading centre to break into short segments and rotate to a $N 17^\circ E$ trend. The small east-west transform faults in the Famous area formed during this change. This new oblique configuration seems to have continued to the present. The maintenance of the oblique relationship through the readjustment in directions shows that it is not a chance occurrence. It is the stable configuration for this spreading system.

Let us review the data which led to these conclusions. The alignment of the presently active transform faults and spreading centre are well documented. In the fracture zones, large, medium, and fine-scale topography as well as microearthquakes all show east-west lineations²⁻⁴. In the spreading centre, the central magnetic anomaly, the large-scale topography, and more than 700 fine-scale tectonic lineations (faults and fissures) mapped in the rift inner floor all have a pronounced $N 17^\circ E$ strike⁵⁻⁹ (Fig.

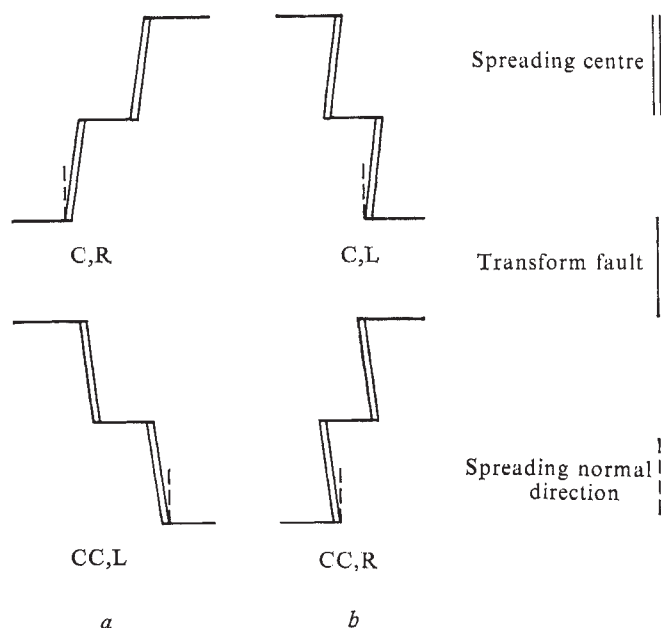


Fig. 1 Spreading centre alignments which result in (a) shortening of the connecting transform faults and (b) lengthening of the transform faults. All measured oblique cases are the type shown in (a), that is transform shortening type (C, R or CC, L). C and CC signify clockwise and counter-clockwise rotations of the spreading centres with respect to the spreading normal directions. R and L signify right and left lateral offsets of the spreading centres by the transform faults.

2). There are a few north-south lineations in the inner floor, but they form part of a nearly symmetrical Gaussian distribution of lineation strikes which has a median of $N 17^\circ E$ and a standard deviation of only 6° (ref. 5). Since the lineation pattern is normally distributed about $N 17^\circ E$, we feel that it is misleading to interpret

Table 1

Location	Angle of obliqueness	Sense of obliqueness*	~ Half spreading rate (cm yr^{-1})	Data type used†	Ref.
Mid-Atlantic Ridge					
11°N (Vema F. Z.)	$9^\circ \pm 1^\circ$	CC,L	1.5	T	14,15
15°N	$7^\circ \pm 5^\circ$	CC,L	1.5	T	16
30°N (Atlantis F. Z.)	$6^\circ \pm 4^\circ$	C,R	1	T	17
37°N (Famous Area)	$17^\circ \pm 3^\circ$	C,R	1	T,M,E	2,3,4,6
40°N (Kurchatov F. Z.)	$38^\circ \pm 5^\circ$	C,R	1	T	17,18
52°N (Charlie-Gibbs F. Z.)	$35^\circ \pm 5^\circ$	CC,L	1	T	19
Gulf of Aden					
48°E	$16^\circ \pm 6^\circ$	CC,L	1	T,M	20
51°E (Alula-Fartak F. Z.)	$18^\circ \pm 7^\circ$	CC,L	1	T,M	21
Juan de Fuca Ridge					
44°N (Blanco F. Z.)	$11^\circ \pm 3^\circ$	CC,L	3	M	22
Gulf of California					
23°N (Tamayo F. Z.)	$1^\circ \pm 3^\circ$	P	3	T,M	23,24
Galapagos Spreading Centre (Panama F. Z.)	$2^\circ \pm 3^\circ$	P	3	T,M,F	25
East Pacific Rise					
9°N (Siquiros F. Z.)	$0^\circ \pm 4^\circ$	P	6	T	‡
3°S (Quebrada F. Z.)	$0^\circ \pm 3^\circ$	P	7.5	T	§
6°S northern	$4^\circ \pm 4^\circ$	P (C,R)	7.5	T,M	26
6°S southern	$4^\circ \pm 4^\circ$	P (CC,R)	7.5		26
N. E. Pacific					
40°N (Mendocino F. Z.)	$0^\circ \pm 2^\circ$	P	3.7	T,M	27,28
34°N (Murray F. Z.)	$-1^\circ \pm 3^\circ$	P	5.0	T,M	28,29

*See Fig. 1 for explanation of symbols; P, perpendicular; F. Z., Fracture Zone.

†T, magnetic anomalies; T, topographic chart; E, earthquake epicentral locations.

‡B. R. Rosendahl and L. Dorman unpublished chart.

§P. F. Lonsdale, data from Pleiades cruise 1976.

the observation of a few north-south lineations as evidence for readjustment to orthogonal spreading as some workers have done. The magnetic maximum along the inner floor axis is caused by crust less than 0.2 Myr old and it also has a N 17° E trend⁹. This is independent evidence that even the youngest crust in the inner floor is created in a direction oblique to the nearest transform faults.

Evidence for the alignment between recent times and 3 Myr, and for the preceding change in direction, comes mostly from the magnetic anomalies (Fig. 3), although their interpretation is supported by lineation of the large-scale ridge crest topography. Deep-tow and surface magnetic data indicate that magnetic anomalies back to 2' trend about N 17° E. Between anomalies 2' and 3' (2.5–5.2 Myr BP) the magnetic lineations rotate so that anomalies 3' and older show a trend of N 35–45° E. (Bird and Phillips¹⁰ report a N 35° E average trend for the anomalies of 0–9 Myr age. It seems that the time interval they used in stacking the anomalies was too large to resolve the change in trend for anomalies less than 3 Myr old.) The magnetic anomalies in Fig. 3 are offset right-laterally back to anomaly 2'. Between anomalies 2' and 3' the anomalies change trend, the offsets decrease and the topographic fracture zones disappear. For anomaly 3' and older, the magnetic lineations are nearly continuous in a N 35–45° E direction. To establish the spreading direction before the change in direction and the creation of the Famous area fracture zones, we must find a larger, more continuous fracture zone. The nearest major one is the Oceanographer, at 35° N. There is some confusion about this feature since its overall trend is N 105° E, not east-west like the smaller fracture zones. We interpret this to be

because the fossil direction, the direction of spreading before the change, and the present east-west motion has not continued long enough to develop a clear readjustment in the gross topography of this long offset fracture zone. Young east-west motion is shown by medium-scale east-west features around the zones^{11,12} and by a tightly constrained focal mechanism solution¹³. Thus, we assume that the present direction of relative plate motion to be east-west while the direction before 5 Myr ago was 15° south of east. The older Famous area anomalies are 20–30° oblique to this direction.

Given this line of reasoning, we conclude the following: on the Mid-Atlantic Ridge at 35–37° N (1) spreading is stably oblique at present; (2) the spreading pattern has had a stable obliqueness of 17° in the present configuration for approximately 3 Myr; (3) spreading was 20–30° oblique in this area before 5 Myr and remained oblique through a change in spreading direction and creation of new fracture zones.

We may have made a mistake in using fracture zones A and B to delineate recent plate motions. In a re-evaluation of global plate tectonic solutions, T. Jordan and B. Minster (personal communication) and others have shown that the north-central Atlantic is one of several problem areas; in particular the east-west relative motion indicated by these transform faults is difficult to reconcile with other plate motions around the Azores triple junction. A more acceptable motion on those grounds would occur if the direction of relative plate motion in the Famous area were somewhat south of east. This implies an oblique opening across the Famous area fracture zones. While detailed deep-tow, microearthquake, and submersible studies^{2–4} indicate that this is not the case, we cannot entirely rule it out. If

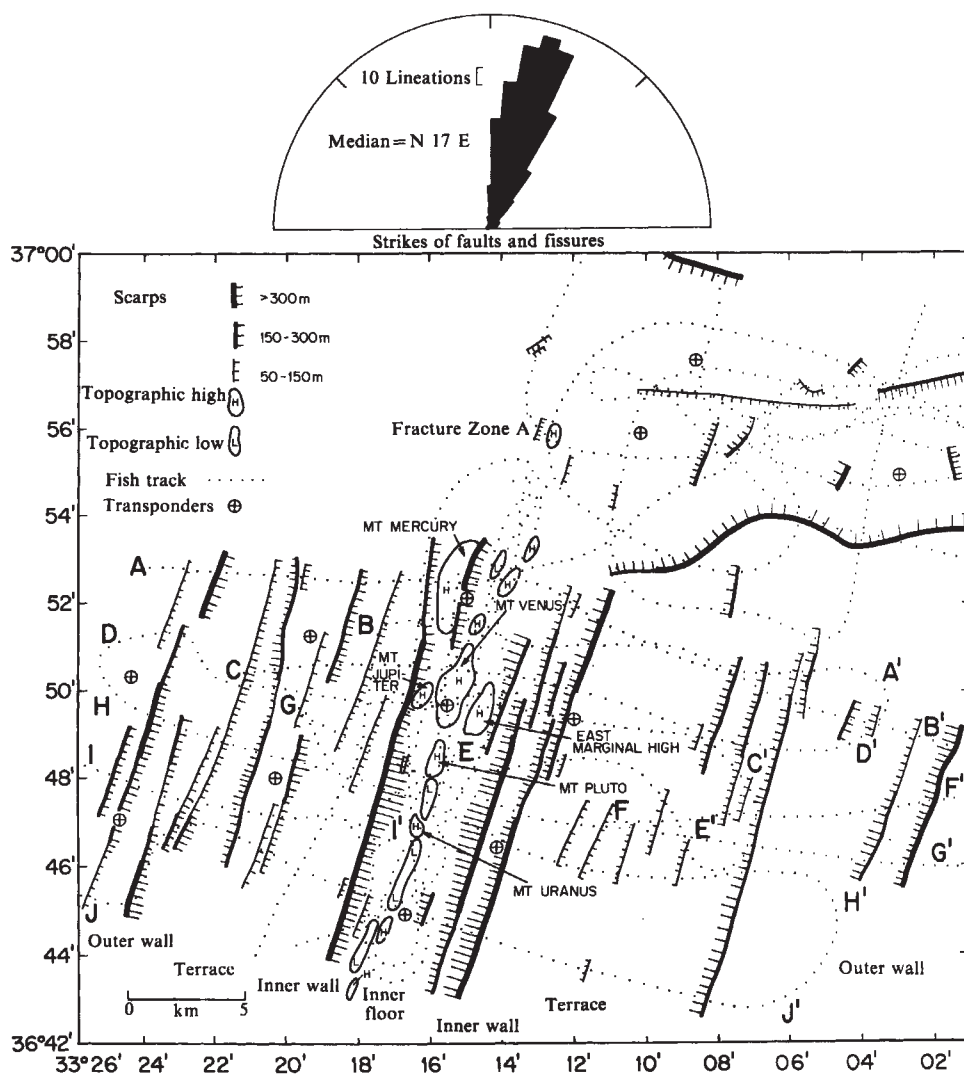


Fig. 2 Tectonic map Famous area showing major scarps of rift valley and Fracture Zone A^{3,6} and rose diagram showing orientation of 100 small lineations in the inner floor and walls⁴. Both large and small features show a N17° E trend for the rift valley. Distinct scarps show an approximate east-west trend for Fracture Zone A.

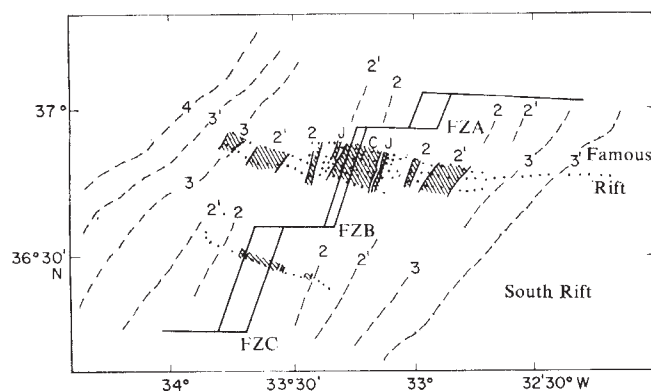


Fig. 3 Sketch map of magnetic lineations in the Famous area⁹ (modified from Phillips and Fleming, in preparation). Hachured areas show extent of magnetic anomalies as detailed by deep-tow survey⁹. Rift valleys and transform faults are shown as mapped from the bathymetry. Note that anomalies younger than 2' are aligned approximately N20° E and are offset by the transform faults while anomalies older than 2' are aligned approximately N40° E and are not significantly offset. The transform faults seem to have originated at the time of the change in direction, between anomalies 2 and 3.

Jordan's preferred direction is assumed, the amount of obliquity for the last 3 Myr is reduced (to 5–10°) but is not eliminated, and it is still in the transform shortening sense.

As we became convinced of the stable oblique spreading in the Famous area, we began to wonder if most 'perpendicular' spreading systems might be somewhat oblique. While no other area of the oceanic ridges is known as thoroughly as the Famous region, we found a number of sections which are well enough mapped either topographically, magnetically, or both to allow measurement of trends. For slowly spreading regions (half-rate less than 3 cm yr⁻¹), we found eight such surveys which include both spreading centres and adjoining transform faults. These are listed in Table 1 with their angles of obliqueness and a subjective estimate of the uncertainty with which the mapped features constrain the trends. As shown all these ridges were found to be oblique, without exception, and, furthermore, every case was oblique in a transform-shortening sense (either C, R or CC, L). Not included in the table is Johnson and Vogt's³¹ chart of the Mid-Atlantic Ridge 47°–50° N. The 500-m contour interval of the chart was too coarse to make precise measurements. Most of the ridge segments, however, seem to be 15°–40° oblique.

For fast spreading centres, only three high resolution, fine-scale maps exist which include both the ridge crest and the adjoining transform fault. These are shown in the Table. In all cases, the transform faults are perpendicular to the spreading centres.

In the survey at 6° S, the transform fault trend is controlled by only two crossings. The magnetic signature of the fault is so sharp, however, that we consider it to be reasonably controlled. Note that the southern intersection at 6° S has an obliquity of 4° in a transform lengthening sense, but within the measurement error, is indistinguishable from perpendicular. Rea²⁶ suggests that the odd trends in the area are due to a recent change in spreading direction with a lag in the topographic adjustment.

Some data exist in other fast spreading areas. Less detailed maps of the Pacific–Antarctic Rise indicate that the angle between the spreading centres and transform faults is never resolvable different from 90° (ref. 29). In the north-east Pacific, an older region of fast spreading has been quite well mapped. The configurations of the ancient ridge crests and transform faults are preserved in the magnetic lineations and fracture zones and these also are perpendicular, as shown in Table 1.

Reasonable surveys exist for three intermediate-rate spreading centres, half-spreading rate around 3 cm yr⁻¹. As shown in Table 1, these give mixed results. The Juan de Fuca Ridge is unquestionably oblique, about 10° in a transform-shortening sense. The other two, the mouth of the Gulf of California and the

Galapagos Ridge, do not seem to be significantly different from perpendicular.

Why do spreading plate boundaries break up into alternating ridge and transform fault segments? Why don't they simply retain the irregular shape which they inherit from the original plate break up? Lachenbruch and Thompson³² and Lachenbruch³³ suggest that the final stable configuration taken by the ridges and transform faults is the one which minimises the mechanical energy dissipated during the accretion and spreading processes. The perpendicular configuration is that which minimises the length of ridge crest in the system at the expense of adding whatever amount of transform fault is needed. Thus, if the minimum energy principle is correct, oblique configuration implies that the energies dissipated by the two features are more comparable. To quantify this comparison, Lachenbruch³³ developed a ratio, S/r_0 , which is approximately equivalent to the ratio between the energy dissipated along the transform fault and that dissipated in the spreading centre. Configurations which are nearly perpendicular like those found at the fast spreading centres, result in a value for S/r_0 near zero. In cases of oblique spreading the relationship between angles and energies is more difficult to evaluate. While transform faults are relatively well understood, the energy dissipated by spreading centres depends on the emplacement model assumed and very few constraints exist for such models. Lachenbruch³³ derives some appropriate equations describing the various likely model types. Using his models and our measured obliquenesses, the S/r_0 ratio ranges from 0.2 to 1.6. In other words, the amounts of energy being dissipated by the transform faults and by the ridges in slow-spreading systems seem to be of about the same magnitude.

The concentration of earthquakes at transform faults shows that they are active dissipators of energy. The above discussion implies, however, that the spreading centres dissipate as much energy as the faults and in many cases much more. This is not very surprising for slowly spreading centres. The rifts at their centres are commonly assumed to be an indication that emplacement of new material at depth is accomplished only with difficulty and thus at the expense of considerable dissipation of energy. Fast spreading centres, however, have a profile which closely approaches that expected from buoyancy in a relaxed state. We would expect the energy dissipation to be less in this case. This presents us with a basic dilemma, since fast ridges are nearly perpendicular, implying a very small value for S/r_0 . If, as we concluded, the energy dissipated by fast spreading centres is small, it follows that the energy dissipation on fast transform faults is very small, and in particular, it is much less than that dissipated on slow transform faults.

This ramification of the minimum energy argument is difficult to accept. It is possible to think of arguments to suggest that slow transform faults might dissipate more energy than fast ones or vice versa. In either case, however, one might expect that the total energy dissipation should be a function of fault length, longer faults dissipating more energy. In fact, the minimum energy argument as interpreted above would suggest that the energy loss on the Siqueiros transform fault (perpendicular configuration, fast rate) must be much less than the loss on transform fault A in the Famous area (oblique, slow rate), even though the former is more than seven times as long as the latter. The conflict would be even greater comparing transform fault A with the fossil offset of the Mendocino transform fault, for example. This leads us to suspect the validity of the minimum energy principle, at least as it is presently formulated.

We conclude, therefore, that all well-studied, segmented, slow spreading centres are oblique to their transform faults, while fast spreading centres seem to be very nearly perpendicular, and intermediate-rate centres are sometimes oblique and sometimes perpendicular. In all cases of significantly nonperpendicular ridges, the obliqueness is in the sense such that the transform faults are shorter than they would be if the ridges were perpendicular. Furthermore, the configurations at given spreading centres seem to be stable for millions of years and through changes in direction of spreading. We believe that these obser-

vations may supply a strong constraint for mechanical models of seafloor spreading and that they raise serious doubts concerning presently accepted models.

We thank the US Office of Naval Research and US NSF for support.

TANYA ATWATER

Department of Earth and Planetary Science,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

KEN C. MACDONALD

University of California,
San Diego Scripps Institution of Oceanography,
La Jolla, California 92093

Received 1 August; accepted 13 October 1977.

1. Vogt, P. R., Avery, O. E., Anderson, C. N., Bracey, D. R. & Schneider, E. D. *Tectonophysics* **8**, 285 (1969).
2. Reid, I. & Macdonald, K. C. *Nature* **246**, 88 (1973).
3. Detrick, R., Mudie, J. D., Luyendyk, B. P. & Macdonald, K. C. *Nature* **246**, 59 (1973).
4. Choukroune, P., Francheteau, J. & Le Pichon, X. (in preparation).
5. Luyendyk, B. P. & Macdonald, K. C. *Geol. Soc. Am. Bull.* **88**, 507 (1977).
6. Macdonald, K. C. & Luyendyk, B. P. *Geol. Soc. Am. Bull.* **88**, 621 (1977).
7. Ballard, R. D. & van Andel, T. H. *Geol. Soc. Am. Bull.* **88**, 507 (1977).
8. Phillips, J. D. & Fleming, H. S. *Geol. Soc. Am. Bull.* (in the press).
9. Macdonald, K. C. *Geol. Soc. Am. Bull.* **88**, 541 (1977).
10. Bird, P. & Phillips, J. D. *J. geophys. Res.* **80**, 4021 (1976).
11. Fox, P. J., Lowrie, A. Jr. & Heezen, B. C. *Deep-Sea Res.* **16** (1969).
12. Fox, P. J., Schroeder, F. W., Moody, R. M., Pitman, W. C. & Hoose, P. J. *Deep-Sea Res.* (in the press).
13. Sykes, L. R. *J. geophys. Res.* **72**, 2131 (1967).
14. van Andel, T. H. *Mar. geophys. Res.* **1**, 274 (1971).
15. Eittreim, S. & Ewing, J. *Geology* **3**, 555 (1975).
16. Collette, B. J. & Ruttner, R. W. *Nature* **237**, 131 (1972).
17. Litvin, V. M., Marova, N. A., Rudenko, M. V. & Udinstev, G. B. *Oceanology* **12**, 631 (1972) (in Russian).
18. Searle, R. C. & Loughton, A. S. *J. geophys. Res.* (in the press).
19. Lonsdale, P. & Shor, A. (in preparation).
20. Loughton, A. S., Whitmarsh, R. B. & Jones, M. T. *Phil. Trans. R. Soc. Lond. A* **267**, 227 (1970).
21. Loughton, A. S. *Phil. Trans. R. Soc. Lond. A* **259**, 150 (1966).
22. Raff, A. D. & Mason, R. G. *Geol. Soc. Am. Bull.* **72**, 1267 (1961).
23. Lewis, B. T. R., McClain, J., Snysman, W. E., Lister, C. R. B., Holmes, M. L. & Heitman, C. *Gulf of Calif. IPOD Site Survey, Final Rep.* (Univ. of Washington, 1976).
24. Macdonald, K. C. & Spiess, F. N. Deep-tow data. F. Drake Cruise (1977).
25. Klitgord, K. D. & Mudie, J. D. *Geophys. J. R. astr. Soc.* **38**, 563 (1974).
26. Rea, D. K. *J. geophys. Res.* **81**, 1495 (1976).
27. Elvers, D., Potter, K., Seidel, D. & Morley, J. NOS Sea Map BGM-1-71, N.O.A.A. (1972).
28. Menard, H. W. in *Marine Geology of the Pacific* 271, (McGraw-Hill, New York, 1964).
29. Mason, R. G. & Raff, A. D. *Geol. Soc. Am. Bull.* **72**, 1259 (1961).
30. Molnar, P., Atwater, T., Mammertick, J. & Smith, S. M. *Geophys. J. R. astr. Soc.* (1976).
31. Johnson, G. & Vogt, P. *Geol. Soc. Am. Bull.* **84**, 3443 (1973).
32. Lachenbruch, A. H. & Thompson, G. A. *Earth planet. Sci. Lett.* **15**, 116 (1972).
33. Lachenbruch, A. H. *J. geophys. Res.* **81**, 1883 (1976).

Seafloor spreading south of the Agulhas Fracture zone

FRANCHETEAU and Le Pichon¹ have suggested that before the break-up of Africa and South America the continental² Falkland Plateau fitted against the southern and south-eastern margin of South Africa from the southern tip of the Agulhas Bank to a position between East London and Durban. During break-up the Plateau acted as part of the South American plate and moved past Africa along a line of shear which was coincident with an initial marginal offset of approximately 610 miles. If the hypothesis is correct and normal spreading took place to the present, the original offset should be preserved in the present-day mid-Atlantic Ridge. In addition, a fracture zone should bound the southern margin of Africa and extend westwards to the mid-Atlantic Ridge, and the Cainozoic³ and Mesozoic⁴ magnetic lineaments that have been traced in the Cape Basin should be present in the Natal Valley, Transkei Basin and northern Agulhas Basin south of the fracture zone, trending perpendicular to it. New magnetic and bathymetric data presented here lend qualified support to the hypothesis and give an indication of the possible seafloor spreading history of the area.

Bathymetric, gravity and magnetic data⁵⁻⁷ have been used to prove the existence and accurately trace the position of the Agulhas Fracture Zone⁸ south of the southern continental margin of South Africa. West of the southern tip of the Agulhas Bank its existence has been proven⁵⁻⁹ but it has hitherto not been accurately traced or linked with a

specific morphological feature on the sea floor. Data from numerous traverses that were run by the RV Thomas B. Davie of the University of Cape Town were used to update the bathymetric map of the South Atlantic of Simpson¹⁰ in the area of the Cape Rise. The new map is presented in Fig. 1. It features a ridge that starts at 23.5°E and extends south-westwards. South of the Agulhas Bank the ridge height is variable along its length; less than 500 m above the surrounding sea floor in places and up to 3,000 m high in others, for example Mallory Seamount. West of the tip of the Agulhas Bank it becomes a low relief feature (<500 m) extending to the Richardson Seamount where it again changes character to become an elevated (>2,000 m) structure which can be followed to 5°E. The total feature is here named the Agulhas Ridge. The 68° small circle about Le Pichon's¹¹ early pole of opening for the South Atlantic has been superimposed on the map and is seen to closely coincide with the trend of the Ridge between 23.5°E and 5°E. This leads to the conclusion that the Ridge accurately reflects the westward extension of the Agulhas Fracture Zone and is the exact counterpart to the linear basement structure that marks the Falkland Fracture Zone between 28°W and 40°W (ref. 9).

There is little suitably orientated magnetic data from south of the Agulhas Fracture Zone. For the purpose of this letter a single composite track, run south of and parallel to the fracture zone from the latitude of Durban to the mid-Atlantic Ridge, was built up from two unpublished traverses of the Geological Survey of South Africa (Pr. 2a and 2b), a short section of traverse from Emery *et al.*⁷ (Pr. 2c) and from traverse 1103 of Ladd *et al.*¹² (Pr. 2d). The insert in Fig. 2 shows the position of their tracks. In Fig. 2 the magnetic profile derived from measurements taken on these traverses (Pr. 2) is compared with a typical profile obtained north of the fracture zone across the Cape Basin between Cape Town and Tristan da Cunha (Pr. 1). The latter profile has been subdivided into four zones for the purposes of this discussion. Zone A includes the anomalies of the Cainozoic sequence terminating in Anomaly 34 (82–85 Myr BP, ref. 13). Following on Anomaly 34 there is a very quiet field defined as Zone B. Zone C includes an initial group of large amplitude anomalies followed by anomalies of shorter wavelength and lesser amplitude. These anomalies have been correlated with the Cretaceous Normal Polarity Epoch by some^{4,8,12} and with the later anomalies of the Mesozoic sequence by others^{7,14}. Zone D includes a set of high amplitude anomalies which Larson and Ladd⁴ named the Cape Basin Lineaments and correlated with anomalies M0 to M12 (~108 to ~128 Myr BP) of the world-wide Mesozoic sequence¹⁵.

In comparing Pr. 2 with Pr. 1 it is seen that a large negative anomaly occurs on Pr. 2 in exactly in the same relative position that Anomaly 34 occurs in Pr. 1 and that though individual intercorrelations are not evident in the 'older than Anomaly 34' crust, a grouping of anomalies may be recognised that is not dissimilar to the grouping in the Cape Basin. Pr. 3 to 6 (Fig. 2) are from four closely spaced sub-parallel traverses which were run in the Natal Valley between the latitudes of East London and Durban. The clear intercorrelation that exists between individual anomalies on Profiles 2a, 3, 4 and 5 confirms that these lineations that may be equivalent with the anomalies in Zone D in Pr. 1 in fact, trend perpendicular to the supposed local transform direction. In summary, the evidence indicates that at 85 Myr BP the original offset in the mid-ocean ridge system of 610 miles was still in existence.

On the 'young side' of Pr. 2 Ladd *et al.*¹² have shown that the present-day mid-Atlantic Ridge is offset by a mere 150 miles in the same sense as the original offset and that the normal Cainozoic sequence is present to at least Anomaly 24 (60 Myr BP) east of the mid-Atlantic Ridge. Beyond 24

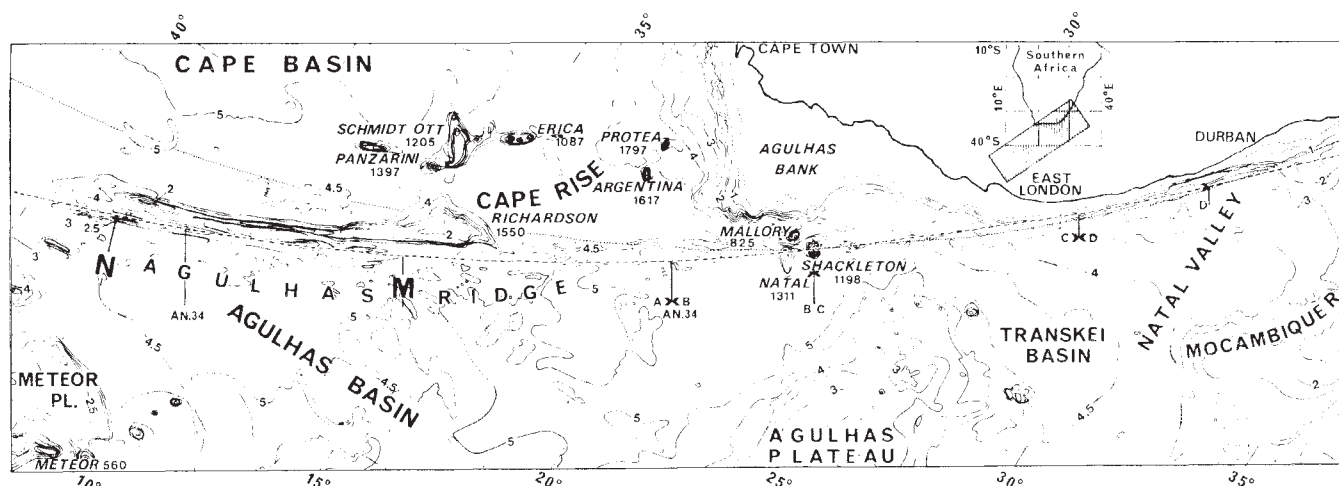


Fig. 1 A bathymetry map with a 500-m contour line interval featuring the extension of the Agulhas Fracture Zone west of South Africa. Insert shows the area covered by the map relative to Southern Africa. The 68° small circle about Le Pichon's¹¹ early pole of opening at 21.5°N , 14°W (stippled line) and the 48.7° small circle about 6°N , 4°W (dotted line) are shown. The first small circle closely approximates the trend of the Agulhas Ridge west of 23.5°E . The second smaller circle is better approximation of the trend of the continental margin east of 23.5°E . The following are also shown: the boundaries between magnetic anomalies groupings A, B, C and D occurring south of the fracture zone which are correlated with a similar grouping of anomalies in the Cape Basin; the positions at which anomaly 34 is traced south of the fracture zone; positions M and N which indicate respectively the positions from which, and to which, the mid-ocean ridge is believed to have jumped at approximately 67 Myr BP.

the traverse crosses the Agulhas Fracture Zone into the Cape Basin. In the absence of any evidence of asymmetrical spreading the simplest mechanism which can be invoked to explain the difference between the present day offset of the mid-Atlantic Ridge and its original offset is a ridge jump of $(610-150) = 460$ miles to the west sometime between 60 Myr (Anomaly 24) and 85 Myr (Anomaly 34) ago. In the central portion of Pr. 2 the data are consistent with the proposed jump having occurred at approximately 67 Myr BP (Anomaly 26): there is an apparent mirroring of magnetic lineaments about the point marked M where the ocean floor is rugged and slightly elevated above the normal level in the Agulhas Basin (Figs 1 and 2). Anomaly 34 occurs both to the east and west of M and so too, do Anomalies 31 and 32 (with less assurance). The position to which the ridge could have jumped is indicated by the point N on Figs 1 and 2.

A number of observations follow on the acceptance of this hypothesis. (1) The Agulhas Plateau is underlain by oceanic crust but is not an extinct spreading centre as suggested by Scrutton¹⁶. Assuming seafloor spreading to have started in the area at 128 Myr BP (ref. 4) the age of the crust which was involved in the tectonism and/or vulcanism

that created the Plateau centres on approximately 95 Myr BP. (2) In the block bounded by latitudes 45° and 50°S and longitudes 0° and 5°E there is an elevated plateau ($\sim 2,500\text{m}$) west of M which overlies crust of similar age to the Agulhas Plateau. It may be the product of the same tectonic event. The plateau is here named the Meteor Plateau (Fig. 1). (3) The trend of the Agulhas Fracture Zone does not exactly correspond with a small circle about Le Pichon's¹¹ early pole of opening east of 23.5°E . The curvature of the small circle is less than the curvature of the fracture zone which has been defined as occurring at the base of the steep continental slope in the area⁶. A better fit is obtained by plotting the 48.7° small circle about a pole of opening at 6°N , 4°W . This lends support to the suggestion of a number of workers^{8,14,17} that during the earliest phase of opening of the South Atlantic spreading was about a pole other than the Le Pichon's early pole. The Agulhas Plateau may reflect the tectonic response of the crust to a readjustment in the spreading direction on the mid-ocean ridge when it reached 23.5°E in the area, 95 Myr ago. (4) M is situated near to the point where the Agulhas Ridge undergoes the dramatic change from a low narrow ridge to a complex elevated structure and N near to the

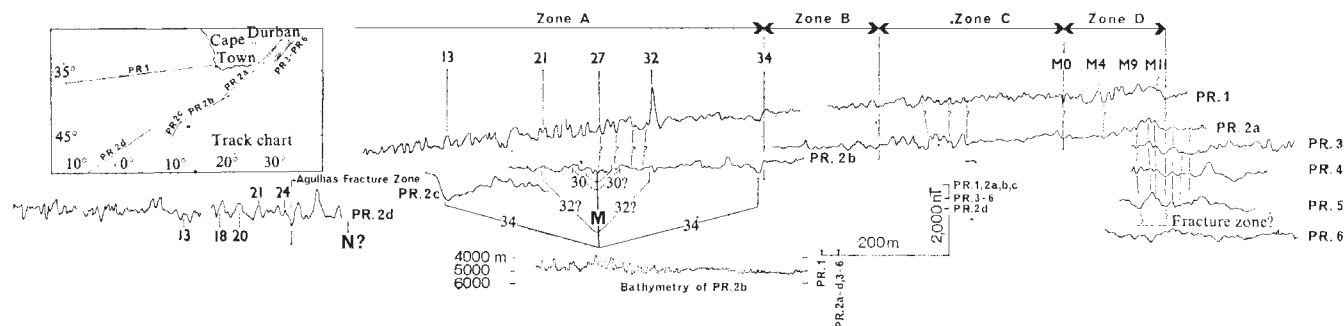


Fig. 2 Total magnetic field profiles plotted perpendicular to transform direction relative to an arbitrary datum and aligned to the first 'apparent' seafloor spreading anomaly adjacent to the continental margin. Map insert gives positions of tracks. For data sources see text. Pr.1 is a typical profile across the Cape Basin with Mesozoic anomaly identifications following Larson and Ladd⁴. Pr.2 is a composite profile from south of the AFZ (Agulhas Fracture Zone). Four groupings of anomalies (Zones A, B, C and D) are shown to occur similarly on Pr.1 and Pr.2. A few tentative individual correlations are shown. Clear intercorrelations between the anomalies on Profiles 2a, 3, 4 and 5 show that the anomalies of Zone D in the Natal Valley trend perpendicular to the AFZ. An apparent symmetrical arrangement of the Cainozoic anomalies 27-34 occurs about point M south of the AFZ. A ridge jump of 460 miles from M to N is postulated (see text). The bathymetry on Pr.2b is shown.

western termination of the Ridge. This apparently suggests that during the period that vulcanism dwindled on the mid-ocean ridge at M, and was increasing at the new position of the ridge at N, it was intense along the Agulhas Fracture Zone mainly between the two points and created the elevated portion of the Agulhas Ridge.

A. DU PLESSIS

Joint Geological Survey/University of Cape Town

Marine Geoscience Unit,

Department of Geology,

University of Cape Town,

Rondebosch, South Africa

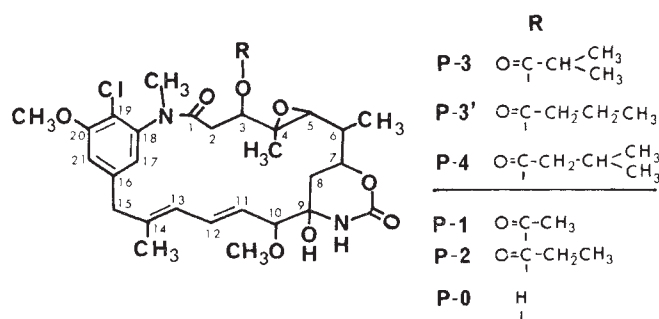


Fig. 1 Structures of ansamitocin and related compounds.

Received 26 September; accepted 20 October 1977.

1. Francheteau, J. & Le Pichon, X. *Bull. Am. Ass. Petrol. Geol.* **56**, 991–1007 (1972).
2. Barker, P. F. *et al. Geotimes* **19**, 16–18 (1974).
3. Dickson, G. O., Pitman III, W. C. & Heirtzler, J. R. *J. geophys. Res.* **73**, 2087–3100 (1968).
4. Larson, R. L. & Ladd, J. W. *Nature* **246**, 209–212 (1973).
5. Talwani, M. & Eldholm, O. *Nature* **241**, 325–330 (1973).
6. du Plessis, A. & Simpson, E. S. W. *Mar. geophys. Res.* **2**, 99–110 (1974).
7. Emery, K. O., Uchupi, E., Bowin, C. O., Phillips, J. & Simpson, E. S. W. *Bull. Am. Ass. Petrol. Geol.* **59**, 2–59 (1974).
8. Rabinowitz, P. D. *Bull. Geol. Soc. Amer.* **87**, 1643–1653 (1976).
9. Rabinowitz, P. D., Conde, S. C. & La Brecque, J. L. in *Continental Margins of Atlantic Type* (ed. de Almeida) *Anais Da Academia Brasileira de Ciencias* **48**, suppl., 241–251 (1976).
10. Simpson, E. S. W. (unpublished map of National Research Institute of Oceanology, Stellenbosch, 1974).
11. Le Pichon, X. *J. geophys. Res.* **73**, 3661–3697 (1968).
12. Ladd, J. W., Dickson, G. O. & Pitman III, W. C. in *The South Atlantic* (eds Nairn, A. E. M. & Stehli, F. G.) (Plenum, New York, 1973).
13. Larson, R. L. & Pitman III, W. C. *Bull. Geol. Soc. Amer.* **83**, 364–366Z (1972).
14. Du Plessis, A. *Bull. Geol. Surv. S. Africa* (in the press).
15. Larson, R. L. & Hilde, T. W. C. *J. geophys. Res.* **80**, 2586–2594 (1975).
16. Scrutton, R. A. *Earth Planet. Sci. Lett.* **19**, 250–256 (1973).
17. Mascle, J. & Sibuet, J.-C. *Nature* **252**, 464–465 (1974).

Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from *Nocardia*

WE have isolated a new group of ansamycin antibiotics with potent antitumour activity, from a fermentation broth of *Nocardia* sp. No. C-15003 (N-1) and have named it ansamitocin. Structures of ansamitocin were found to be similar to maytansinoid and related maytansinoids obtained from plant sources by Kupchan *et al.*^{1–4} and Wani *et al.*⁵. These compounds have strong antitumour activities, but development of production would be difficult, because plants containing maytansinoids are only harvested in tropical areas and their content in the plants is extremely low. Some attempts have been made to find a maytansinoid-producing microorganism⁶, but no success has been reported. We report here the microbial production, isolation and structural elucidation of these antibiotics and their antitumour activities against several experimental tumours in mice.

Strain No. C-15003 (N-1), which forms many coraemia-like bodies and motile heteromorphous cells was concluded to be a new species of the genus *Nocardia*. Fermentation was carried out at 28 °C for 4 d under aeration and with agitation, in a 2,000-l

fermentor containing 1,000 l of a culture medium consisting of 5% dextrin, 3% corn steep liquor, 0.1% peptone and 0.5% CaCO₃. In the culture conditions, most of the active substances were produced in the extracellular medium.

The active substances were extracted with ethyl acetate from the culture filtrate. The extract was chromatographed on a silica gel column using a mixture of CHCl₃ MeOH and aqueous ethyl acetate to give five crystalline products, P-1, P-2, P-3, P-3' and P-4, of which P-3 and P-4 were the most abundant. The physicochemical properties of these antibiotics are as follows: P-1, C₃₀H₃₉ClN₂O₉, m.p. 235–236 °C, $[\alpha]_D^{25} - 121^\circ$ ($c=0.25$ in CHCl₃), MS m/e 545 ($M^+ - a^*$), P-2, C₃₁H₄₁ClN₂O₉, m.p. 188–190 °C, $[\alpha]_D^{25} - 127^\circ$ ($c=0.35$ in CHCl₃), MS m/e 559 ($M^+ - a^*$); P-3, C₃₂H₄₃ClN₂O₉, m.p. 190–192 °C, $[\alpha]_D^{25} - 136^\circ$ ($c=0.375$ in CHCl₃), ultraviolet spectrum $\gamma_{\text{max}}^{\text{MeOH}}$ nm (ϵ) 233 (30,250), 240 (sh. 28,450), 252 (27,640), 280 (5,750), 288 (5,700), PMR (100 MHz in CDCl₃) δ p.p.m. 1.27 (d. 3H, $-\text{CH}-\text{CH}_3$), 1.28 (d. 3H, $-\text{CH}-\text{CH}_3$), MS m/e obs. 573.2471 (calc. 573.2493) ($M^+ - a^*$); P-3', C₃₂H₄₃ClN₂O₉, m.p. 182–185 °C, $[\alpha]_D^{25} - 134^\circ$ ($c=0.11$ in CHCl₃), PMR (100 MHz in CDCl₃) δ p.p.m. 1.05 (t. 3H, $-\text{CH}_2-\text{CH}_3$), MS m/e 573 ($M^+ - a^*$); P-4, C₃₃H₄₅ClN₂O₉, m.p. 177–180 °C, $[\alpha]_D^{25} - 142^\circ$ ($c=0.52$ in CHCl₃), PMR (100 MHz in CDCl₃) δ p.p.m. 1.03 (d. 6H, $-\text{CH}(\text{CH}_3)_2$), MS m/e obs. 587.2626 (calc. 587.2649) ($M^+ - a^*$). The ultraviolet and infrared spectra of these antibiotics resemble each other, and no microbial product similar to these antibiotics has been reported previously. All of these antibiotics contain two atoms of nitrogen and one atom of chlorine per molecule, and their ultraviolet spectra resemble that of maytanacin⁴ obtained from a plant source.

Alkaline hydrolysis of P-1, P-2, P-3, P-3' and P-4 gave acetic, propionic, isobutyric, butyric and isovaleric acids, respectively. Analysis of the PMR spectrum and spin-decoupling studies of P-3 demonstrate that the skeletal structure is the same as that of maytansine¹.

Reductive hydrolysis of each antibiotic with LiAlH₄ at lower temperature gave maytansinol (P-O) (ref. 4), and acetylation of P-O with acetic anhydride in pyridine yielded P-1, maytanacin⁴.

In the analysis of mass spectrometry, all of these antibiotics gave the same characteristic fragment peaks at m/e 485, 470 and 450 as

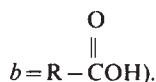
Table 1 Antitumour activity of ansamitocin P-3 and P-4 against mouse leukaemia P388*

Dose ($\mu\text{g kg}^{-1}$ per injection)	Ansamitocin P-3		Ansamitocin P-4	
	MST (d)	ILS† (%)	MST (d)	ILS† (%)
50	13.0	18	13.8	25
25	24.5	123	21.5	95
12.5	19.5	77	19.5	77
6.3	18.5	68	19.8	80
3.2	17.5	59	16.0	45
1.6	15.8	44	15.5	41
0.8	13.5	23	14.5	32
0.4	11.5	5	11.5	5

* (C57BL/6 × DBA/2)F₁ female mice weighing 18–22 g were inoculated intraperitoneally (i.p.) with 10⁶ P388 cells and the test groups each consisting of five mice were treated i.p. with ansamitocin dissolved in methanol–0.85% physiological saline daily for 9 d, starting 24 h after tumour inoculation. The medium survival times (MST) of the test groups (T) were compared with that of the control group (C) consisting of 15 mice (MST = 11.0).

† Percentage increase in lifespan over controls ($(T/C) \times 100$).

those of maytansinoids²⁻⁴: 485 [M⁺ - *(a+b)], 470 [485-15(CH₃)], and 450 [485-35(Cl)]; *(a = H₂O + HNCO,



The MS spectra of P-1 and P-2 were identical to those of maytanacine and maytansinol propionate, respectively. We therefore concluded that P-3, P-3' and P-4 were novel ansamycin antibiotics. The structures of P-3, P-3' and P-4 correspond to those of unknown maytansinoids, maytansinol isobutyrate, -butyrate and -isovalerate, respectively. Figure 1 shows the chemical structures of ansamytocin and their related compounds.

Ansamitocin shows strong growth inhibitory activities against phytopathogenic fungi, dermatophytes and protozoa, but no activity against bacteria.

Antitumour activity of ansamitocin against leukaemia P388 was assayed by the method of Geran *et al.*⁷. As shown in Table 1, P-3 and P-4 had strong antitumour activity against P388 at daily doses as low as 0.8-25 µg kg⁻¹ with a maximum effect at 25 µg kg⁻¹. The wide effective dose range of ansamitocin was very similar to that of maytansine⁸. Antitumour effects of ansamitocin on other experimental tumours were also investigated. In the same daily dose range as that used in P388 test system, P-3 and P-4 were significantly active against B16 melanoma, sarcoma 180, Ehrlich carcinoma and P815 mastocytoma, but less active against leukaemia L1210. Typical figures of mitotic arrest of various ascites tumour cells of mice were observed microscopically 4-9 h after a single injection of these antibiotics. In accordance with this morphological observation of mitotic arrest, P-3 and P-4, like maytansine⁹⁻¹², completely inhibited the polymerisation *in vitro* of tubulin purified from bovine brain^{13,14} at a concentration of 4 µg ml⁻¹ (S. Ikegami and M. Takeuchi, unpublished).

It should be emphasised that the discovery of a microorganism producing ansamitocin opens the way for their production by fermentation. Also, the supply of ansamitocin with its potent antitumour activity may contribute to the research on cancer chemotherapy.

We thank Drs E. Ohmura, M. Isono and Z. Suzuki for their continued interest and fruitful discussions throughout this work. Leukemia P388 was obtained in 1975 from the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research by courtesy of Drs Y. Sakurai and S. Tsukagoshi.

EIJI HIGASHIDE
MITSUKO ASAI
KOICHIRO OOTSU
SEIICHI TANIDA
YOSHIO KOZAI
TORU HASEGAWA
TOYOKAZU KISHI
YUKIO SUGINO
MASAHICO YONEDA

Central Research Division,
Takeda Chemical Industries Ltd,
Juso, Yodogawa-ku, Osaka 532, Japan

12. Wolpert-DeFilippes, M. K., Bono, V. H. Jr, Dion, R. L. & Johns, D. G. *Biochem. Pharmacol.* **24**, 1735-1738 (1975).
13. Shelanski, M. L., Gaskin, F. & Cantor, C. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 765-768 (1973).
14. Gaskin, F., Cantor, C. R. & Shelanski, M. L. *J. molec. Biol.* **89**, 737-758 (1974).

Radiographic thin-section image of the human wrist by nuclear magnetic resonance

We present here a detailed image showing the distribution of mobile protons in a thin section through a human wrist. The image was produced by nuclear magnetic resonance (NMR) techniques. The image consists of 128 by 128 independent picture elements and has a resolution of about 0.4 mm. For the first time images produced by NMR can be compared in quality to those produced by X-ray tomography.

The addition of carefully controlled inhomogeneous magnetic fields makes it possible to perform NMR measurements at selected regions of heterogeneous samples and to form two-dimensional maps of the distribution of almost any property measurable by NMR¹⁻⁶. The possibility of producing two-dimensional NMR images was first demonstrated by P. C. Lauterbur who used the word *zeugmatography* to denote the general method of obtaining spatial information by observing the response of a sample to a controlled inhomogeneous environment¹. We have recently developed a technique for making the NMR spectrometer sensitive to a selected region of the sample³⁻⁶. By applying time-dependent magnetic fields to all of the sample except the selected region and by applying intense rf pulses and then signal averaging, we observe the signal that arises only from the selected region.

The image in Fig. 1 was produced by a combination of techniques. Two uniform orthogonal field gradients with differing time dependence were applied to the sample. The null planes of the two gradients intersect producing a line where the field is static⁴. This line is the selected region to which the spectrometer is sensitive. A third, static, gradient was applied along this sensitive line. The Fourier transform of the resulting NMR signal gives the distribution of the sample along the line. This distribution is displayed as beam

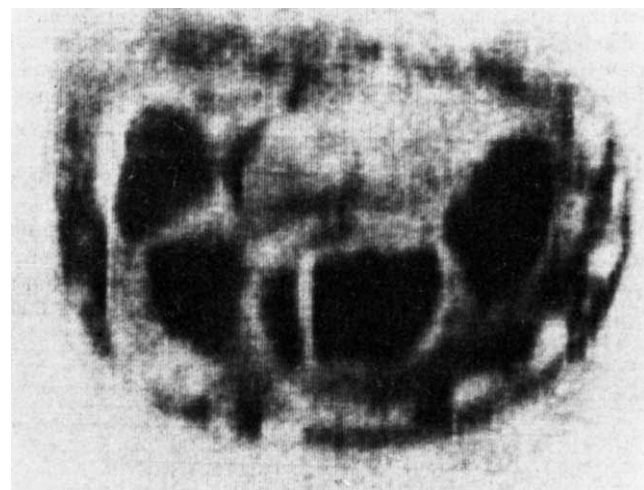


Fig. 1 An NMR image of the distribution of mobile protons in a thin transverse section through the left wrist of one of the authors (P.A.B.). The image is orientated as though the author were facing you with palm upward and was taken at the level of the distal tip of the anterior horn of the lunate. The thumb was held close to the palm causing the general outline to differ slightly from the drawing in Fig. 2. Dark areas in the image indicate regions that contain high concentrations of mobile protons such as the marrow in the carpals and subcutaneous fat. Light areas indicate the presence of tissue with few mobile protons such as tendons, nerves and solid bone. Blood in the veins and arteries is light due to its motion during the imaging process.

Received 9 August; accepted 25 October 1977.

1. Kupchan, S. M. *et al.* *J. Am. chem. Soc.* **94**, 1354-1356 (1972).
2. Kupchan, S. M., Komoda, Y., Thomas, G. J. & Hintz, H. P. *J. chem. Soc., chem. Commun.* 1065 (1972).
3. Kupchan, S. M., Komoda, Y., Brannman, A. R., Dailey, R. G. Jr & Zimmerly, V. A. *J. Am. chem. Soc.* **96**, 3706-3708 (1974).
4. Kupchan, S. M. *et al.* *J. Am. chem. Soc.* **97**, 5294-5295 (1975).
5. Wani, M. C., Taylor, H. L. & Wall, M. E. *J. chem. Soc., chem. Commun.* 390 (1973).
6. Hanka, L. J. & Burnett, M. S. *Antimicrob. Agents Chemother.* **6**, 651-652 (1974).
7. Geran, R. I., Greenberg, N. H., Macdonald, M. M., Schumacher, A. M. & Abbott, B. J. *Cancer chemotherapy, Rep.* **3**, 1-103 (1972).
8. Venditti, J. M., Wolpert-DeFilippes, M. K., in *Chemotherapy* **7**, 129-147 (Plenum, New York and London, 1976).
9. Remillard, S., Rebhun, L. I., Howie, G. A. & Kupchan, S. M. *Science* **189**, 1002-1005 (1975).
10. Schnatman, T., Rebhun, L. I. & Kupchan, S. M. *J. Cell Biol.* **67**, 388a (1975).
11. Wolpert-DeFilippes, M. K., Adamson, R. H., Cysyk, R. L. & Johns, D. G. *Biochem. Pharmacol.* **24**, 751-754 (1975).

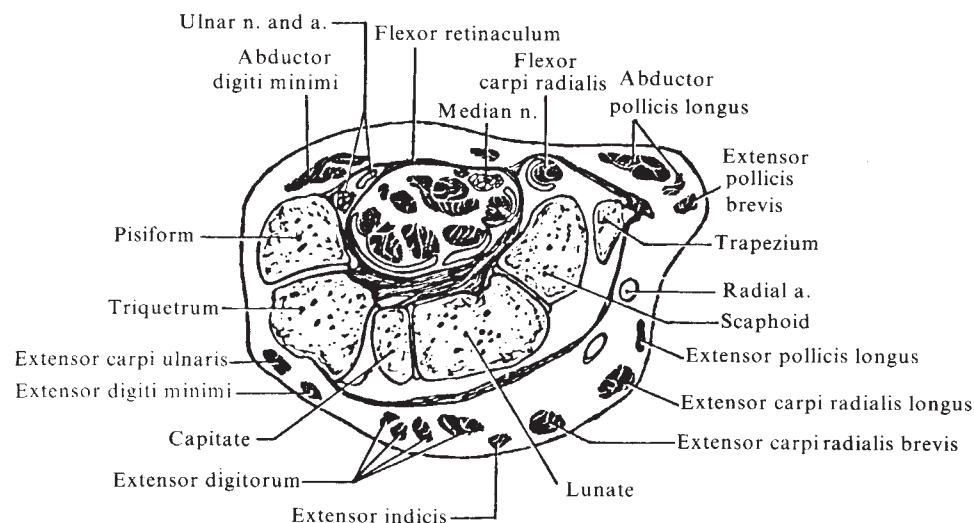


Fig. 2 An illustration from an anatomy textbook which shows many of the features visible in the image. This drawing represents a slightly more distal plane than the NMR image which does not show the trapezium and shows the hamate in place of the capitate. The figure is reprinted from page 141 of ref. 12 with kind permission of the authors and publishers.

intensity along a corresponding line on a storage oscilloscope. Scanning the sensitive line slowly through the sample whilst simultaneously moving the display line results in the formation of a two-dimensional image on the oscilloscope screen.

In producing Fig. 1, the spectrometer settings were such that the solid-like or rigidly-bound protons were not observed whilst those undergoing rapid thermal motion, as in 'free water', were observed. The distribution of mobile protons in Fig. 1 can be correlated with the expected structure as shown in Fig. 2. The image was recorded using a resonance magnetic field of 7 kG which gave a proton resonant frequency of 30 MHz. The two orthogonal field gradients which defined the sensitive line had a sinusoidal time dependence with frequency of ~ 30 Hz and were in phase quadrature. The intensity of all three gradients was between 1 and 10 G cm⁻¹. The phase-alternated 90° rf pulses were 15 μ s long and were applied every 1.9 ms. The sensitive line was ~ 0.4 mm in width and moved by 0.4 mm every 2.5 s thus scanning the object in a total of 9 min. The thickness of the slice was ~ 3 mm.

The ability to add spatial control to NMR measurements provides a tool with considerable potential, particularly in the areas of biological and clinical research and perhaps medical diagnosis. NMR is a well established and powerful analytical technique which is capable of providing detailed information at the molecular level. In principle, localised measurements can be made of properties such as NMR relaxation times T_1 and T_2 , diffusion, flow, and chemical shift. We have been able to record the high resolution chemical shift spectra of the ¹H and ¹⁹F resonances at small selected regions of the sample. The fact that NMR has no known associated hazard increases the potential of the new technique. No permanent effect has been observed from magnetic fields⁷ and the radio frequency irradiation is several orders of magnitude less than that used for diathermy.

Spatially controlled NMR techniques should be applicable to many medical problems. Although recent measurements⁸⁻¹⁰ on excised tissue have given only qualified support to the hope that NMR relaxation behaviour can be used to detect tumours, it is probable that with continued research, NMR parameters can be related to disease states. Oedema and ischaemia have been shown to affect relaxation times. One possible medical application is the imaging of fluorine in order to follow the changing chemical behaviour and physical distribution of fluorine compounds¹¹. Although fluorine has a very low abundance in biological samples, fluorine-bearing drugs can be introduced as tracers. Further applications are also suggested by the recent use of fluoro-

carbons as blood substitutes and as breathing media in small animals.

We wish to express our gratitude to the other members of the group involved in this research, particularly Professor E. R. Andrew and Dr W. S. Moore. The project is supported by a grant from the MRC.

W. S. HINSHAW
P. A. BOTTOMLEY
G. N. HOLLAND

Department of Physics,
University of Nottingham,
Nottingham, UK

Received 10 August; accepted 15 November 1977.

1. Lauterbur, P. C. *Nature* **242**, 190-191 (1973).
2. Lauterbur, P. C. *Pure appl. Chem.* **40**, 149-157 (1974).
3. Hinshaw, W. S. *Phys. Lett.* **48A**, 87-88 (1974).
4. Kumar, A., Welti, D. & Ernst, R. R. *J. mag. Res.* **18**, 69-83 (1975).
5. Hinshaw, W. S. *J. appl. Phys.* **47**, 3709-3721 (1976).
6. Mansfield, P. & Maudsley, A. A. *Br. J. Radiol.* **50**, 188-194 (1977).
7. Barnothy, M. F. (ed.) *Biologic Effects of magnetic Fields* (Plenum, New York), Vol. 1 (1964), Vol. 2 (1969).
8. Knispel, R. R., Thompson, R. T. & Pintar, M. M. *J. mag. Res.* **14**, 44-51 (1974).
9. Hazlewood, C. F., Cleveland, G. & Medina, D. J. *natn. Cancer Inst.* **52**, 1849-1853 (1974).
10. Hollis, D. P., Saryan, L. A., Eggleston, J. C. & Morris, H. P. *J. natn. Cancer Inst.* **54**, 1469-1472 (1975).
11. Holland, G. N., Bottomley, P. A. & Hinshaw, W. S. *J. mag. Res.* (in the press).
12. Gardner, E., Gray, D. T. & O'Rahilly, R. *Anatomy: A Regional Study of Human Structure* (Saunders, Philadelphia, 1975).

Developmental compartments in the proboscis of *Drosophila*

A DEVELOPMENTAL compartment¹ in *Drosophila* is a precisely defined region of the adult fly formed by cells of a polyclone². A polyclone consists of all the descendants of a single group of founder cells set apart at a specific stage during development. Each thoracic appendage is formed by an anterior and posterior compartment^{1,3,4} which descend from nearby groups of blastoderm cells^{5,6}. Within any part of the thorax, the boundary between these compartments does not coincide with an obvious cuticular boundary.

I have studied lineage relationships between cells forming the proboscis, a part of the adult head, by clonal and gynandromorph analysis. The results obtained indicate that each half of the proboscis (left and right) is formed from two compartments that descend from neighbouring groups of blastoderm cells. This finding suggests that the development of the proboscis may be homologous to that of the thoracic appendages, and supports the contention that compartments are a general feature of *Drosophila* development.

Proliferating cells of *Drosophila* embryos and larvae heterozygous for a dominant *Minute* mutation divide at slower rates than cells of wild-type larvae^{7,8}. Single cells

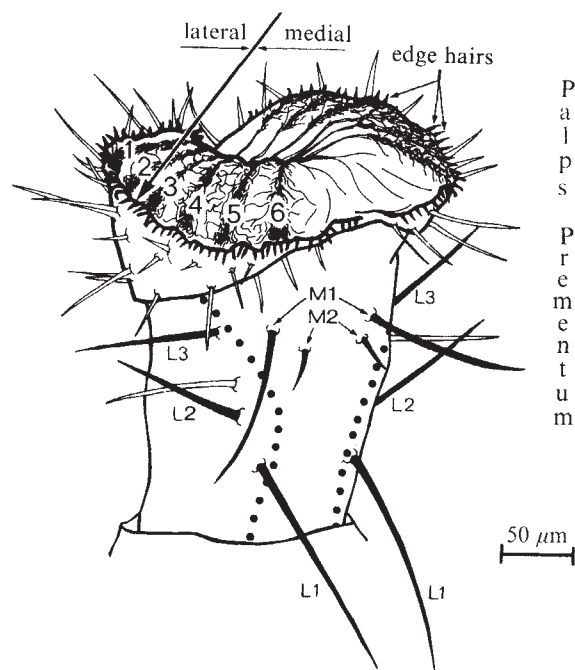


Fig. 1 The posterior aspect of an extended proboscis. The boundary between lateral and medial compartments is indicated by a dotted line on the prementum and by an arrow on the row of edge hairs on the labial palps. The boundary on the exterior surface of the palps is not defined since *javelin* bristles could not be reliably scored there. The six pseudotracheae have been numbered in the anterior to posterior direction. Landmark bristles (blackened in) on the prementum are as follows: the most proximal lateral bristle (L1), the next proximal (L2), the most distal lateral bristle (L3), the largest, most distal medial bristle (M1), and the medial bristle between M1 and the midline (M2). Although up to six lateral and four medial bristles may be present on each half prementum, the landmark bristles can be distinguished by their morphology and position. Nomenclature as in ref. 16. ($\times 200$).

homozygous for the *Minute*⁺ allele can be generated by X ray-induced somatic recombination. Such *Minute*⁺ cells divide autonomously at wild-type rates⁷ and, if made sufficiently early, form clones which fill most of the compartment in which they occur¹. By examining several clones made at a specific point in development, the boundaries demarcating a compartment can be defined precisely¹.

Minute⁺ clones marked with *yellow*, *javelin*, and *multiple wing hairs*⁸ were induced during the cellular blastoderm stage and detected on the proboscis by screening for *yellow javelin Minute*⁺ bristles on the prementum (Fig. 1). All clones of more than one bristle were subsequently screened under the scanning electron microscope to determine the extent of hairs with *multiple wing hairs* phenotype. It is important to note that the proboscis is formed by the fusion

late in development of a left and a right imaginal disk. Since these imaginal disks come from distant blastoderm primordia, each half of the proboscis has been treated as a separate entity.

Table 1 summarizes the classes and frequencies of clones obtained. Of a total of 21 clones marking at least two bristles on the prementum, 12 marked only lateral bristles, nine only medial bristles, and none marked both lateral and medial bristles (Fig. 1). Scanning electron microscopy of the hairs of these marked probosces revealed a precisely defined line separating the region containing lateral bristle clones, from that containing medial bristle clones on the prementum (Figs 1, 2a). All but one clone ran along at least part of this line and 12 of 21 defined it entirely. No clones transgressed this line. In 15 cases, these clones also marked bristles and edge hairs on the labial palps (Fig. 1). The boundary separating the domains of medial and lateral prementum clones was precisely defined along the row of edge hairs. It falls between the second and third pseudotrachea (Figs 1, 2b). Six of nine lateral clones and four of six medial clones which marked the edge hairs reached this boundary and none transgressed it. The boundaries defined on the prementum and the edge hairs of the palps do not coincide with any obvious feature of the cuticle (Fig. 2a, b).

Table 2 Sturt distances between five landmark bristles on the prementum

	M1	M2	L1	L2	L3
M1	—				
M2	0.5	—			
L1	7.8	7.2	—		
L2	7.3	6.5	1.5	—	
L3	7.5	6.6	0.5	1.5	—

Heads of 250 *y w sn³ f^{88a}/In(1) X^{c2} w^{vc}* (ref. 9) mosaic flies were mounted directly in Euparal (GBI Laboratories, Manchester) between two coverslips and scored for the phenotype of each of five landmark bristles on the prementum (Fig. 1), and several landmark bristles on the head. The distances between medial and lateral landmark bristles are boxed. All distances between head and proboscis landmark bristles fell within 29–35 sturts, and those between left and right proboscis landmark bristles within 23–25 sturts. The frequencies with which any of the proboscis or head landmark bristles were male fell between 55–64%.

The domains of both classes of clones account for the entire markable surface of the proboscis. In addition, the majority of clones filled or almost filled the entire domain in which they occurred. Therefore, the failure of these clones to transgress the defined boundaries indicates that each half of the proboscis is formed from two compartments. Since the clones were induced at the blastoderm stage, these compartments probably descend from two groups of founder cells set apart during or soon after this stage.

Although the two imaginal disks that form the proboscis are separate during the blastoderm stage and larval development, they may have come into contact during the intervening period of embryogenesis. If such contact occurs, *Minute*⁺ clones should frequently mark large portions of the same compartment in both left and right proboscis halves. For example, in both anterior and posterior compartments of the first leg, the frequency of left-right overlap was greater than 30% when *Minute*⁺ clones were induced during the blastoderm stage³. Therefore, the behaviour of each of the 21 proboscis clones with respect to the midline was examined.

All the medial clones extended to the midline of the prementum and, with one exception, did not cross it. The exceptional clone marked only one bristle and a region of hairs on the second half prementum. Similarly, of the

Table 1 Head and proboscis clone frequencies

X-ray dose	No. of sides (left and right) screened	No. of clones having more than one marked bristle		
		Proboscis lateral	Proboscis medial	Head
500R	3,796	12	9	28
0	1,014	1	0	0

y; *Dp* (1;3) *sc¹⁴*, *y⁺* *M*(3)*i⁸⁸/mwh jv* (ref. 9) embryos were irradiated during the cellular blastoderm stage (3.0 $\pm$ 0.7 h after egg laying at 25°C) with 500R of X rays (250 KV at 15 mA half-value layer 2.3 mm copper distance 3 cm, rate 53OR min⁻¹). Groups of 50–100 flies were screened blind with similar batches of unirradiated siblings. Clones on the proboscis and head were detected under the dissecting microscope ($\times 50$) by the presence of *yellow javelin Minute*⁺ bristles.

clones that marked the edge hairs, four of six medial and six of nine lateral clones extended to the midline but none crossed it. These results indicate that left and right proboscis primordia are separate until late in development. The sole case of left-right overlap may have been caused by independent clones in the left and right proboscis primordia.

To determine the proximity of the two founder groups of each half proboscis at the blastoderm stage, the distances between blastoderm cells giving rise to lateral and medial landmark bristles on the prementum were estimated by gynandromorph mapping. In this technique, the frequency (in units of 1 sturt=1% of all gynandromorph cases¹⁰) with which two adult structures are of different sex is directly related to the distance separating the blastoderm cells from

which they are derived¹¹. Since neighbouring adult landmarks in the same compartment can descend from the same blastoderm cell, the frequency with which they are of different sex may be extremely low. However, adjacent adult landmarks which descend from different groups of blastoderm cells cannot descend from the same cell; consequently, there must be a lower limit on the sturt distances between them. This lower limit probably represents the distance between adjacent blastoderm cells in different groups of founder cells⁶.

The sturt distances between each of five landmark bristles on the prementum (Fig. 1) are shown in Table 2. The distance between any lateral and any medial bristle is between six and eight sturts. The same range is observed between neighbouring adult landmarks in adjacent thoracic and abdominal compartments^{5,6,12,13} and probably represents the distance between adjacent, but developmentally separate, blastoderm cells⁶. In contrast, the distance between any two lateral or medial bristles is less than two sturts, indicating that they frequently descend from the same blastoderm cell.

I conclude that each half of the proboscis is formed from two developmental compartments that descend from nearby (probably adjacent) groups of blastoderm cells. These compartments appear similar in all respects examined to the anterior and posterior compartments of the thoracic appendages. If a genuine homology exists, homeotic genes such as *engrailed* (which is required for normal development of the posterior compartments in the thorax^{14,15}) should be similarly required in the developing proboscis.

I thank Dr P. A. Lawrence for advice and encouragement. I am supported by a Graduate Fellowship from the National Science Foundation.

G. STRUHL

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge, UK

Received 9 September; accepted 25 October 1977.

1. Garcia-Bellido, A., Ripoll, P. & Morata, G. *Nature new Biol.* **245**, 251-253 (1973); *Devl Biol.* **48**, 132-147 (1976).
2. Crick, F. H. C. & Lawrence, P. A. *Science* **189**, 340-347 (1975).
3. Steiner, E. *Wilhelm Roux' Archiv.* **180**, 9-30 (1976).
4. Morata, G. & Garcia-Bellido, A. *Wilhelm Roux' Archiv.* **179**, 125-143 (1976).
5. Garcia-Bellido, A. & Ferrus, A. *Wilhelm Roux' Archiv.* **178**, 337-340 (1975).
6. Lawrence, P. A. & Morata, G. *Devl Biol.* **56**, 40-51 (1977).
7. Morata, G. & Ripoll, P. *Devl Biol.* **42**, 211-221 (1975).
8. Ferrus, A. *Genetics* **79**, 589-599 (1975).
9. Lindsley, D. L. & Grell, E. H. *Carnegie Inst. & Wash. Publ.* No. 627 (1968).
10. Hotta, Y. & Benzer, S. *Nature* **240**, 527-535 (1972).
11. Garcia-Bellido, A. & Merriam, J. R. *J. exp. Zool.* **170**, 61-75 (1969).
12. Wieschaus, E. & Gehring, W. *Wilhelm Roux' Archiv.* **180**, 31-46 (1976).
13. Lawrence, P. A., Green, S. & Johnston, P. *J. Embryol. exp. Morph.* (in the press).
14. Morata, G. & Lawrence, P. A. *Nature* **255**, 614-617 (1975).
15. Lawrence, P. A. & Morata, G. *Devl Biol.* **50**, 321-337 (1976).
16. Ferris, G. F. in *Biology of Drosophila* (ed. M. Demerec.) 368-418 (Wiley, New York, 1950).

Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle

THE muscular dystrophies are a group of hereditary disorders manifested by a progressive wasting of the skeletal muscles. In spite of extensive studies, the nature of the primary lesion is unknown (for review see ref. 1). Because of the complex interaction between tissues, it is difficult to study this question *in vivo*. Therefore attempts have been made to investigate this question in cultures of dystrophic muscles of human or animal origin. Tissue explants as well as monolayer primary cell cultures contain, in addition to the myogenic cells, a heterogeneous cell population, the composition of which might differ in normal and dystrophic muscle cultures. It is difficult in such experiments to distinguish between properties intrinsic to the myogenic cells and effects exerted by other cell types. Indeed, previous experiments have yielded conflicting conclusions²⁻⁶. We

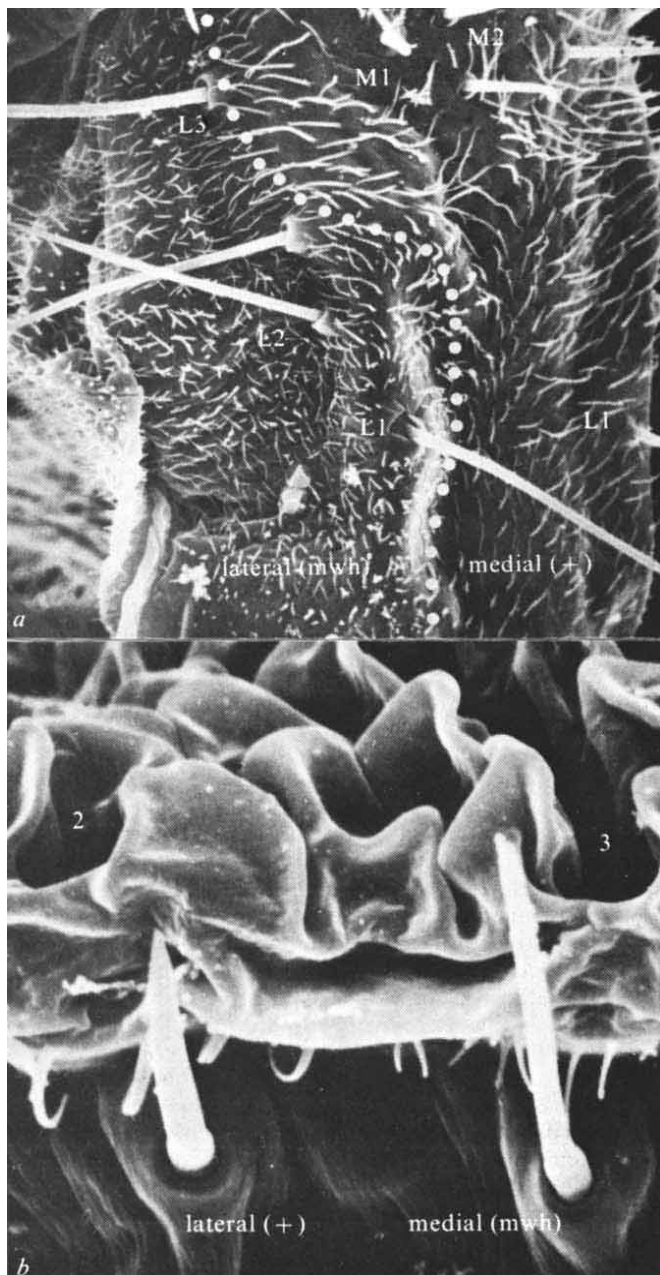


Fig. 2 Scanning electron micrographs of *Minute*⁺ *mwh* clones which define the compartment boundary *a*, on the prementum (500 $\times$); *b*, along the edge hairs of the labial palps (3,200 $\times$). The compartment boundaries, landmark bristles and pseudo-tracheae are indicated as in Fig. 1. Specimens were critical-point dried from absolute alcohol, coated with ~ 30 μ m of gold, and photographed in the Cambridge S4 Stereoscan electron microscope of the Zoological Department, Cambridge.

therefore tested the possibility of obtaining cell cultures consisting of pure populations of myogenic cells obtained from dystrophic muscles. The present report describes the isolation of a cloned population of such cells, derived from adult dystrophic mouse muscle, that can proliferate and differentiate in cell culture.

Mice of strain 129 ReJ homozygous for the recessive gene *dy* were used for the preparation of cultures. These mice manifest a syndrome very similar to human muscular dystrophy which starts early in life and progresses rapidly⁷. Dystrophic thigh muscles of 1–2-month-old mice were excised. After a few pieces had been removed for histological examination, the muscles were cut with scissors into pieces ~ 2 mm in size. A suspension of mononucleated cells was prepared by digestion with a mixture of 0.24% trypsin and 0.1% collagenase in Ca^{2+} -, Mg^{2+} -free phosphate-buffered saline, as described by Yasin *et al.*⁸. Mass cultures were plated at densities of 5×10^4 – 5×10^5 cells per 60-mm gelatin-coated plate, and grown in Dulbecco-modified Eagle's medium supplemented with 20% foetal calf serum⁹. Enrichment for myoblasts was accomplished by selective serial passage of the cultures (before cell fusion), based on the observation that the attachment of suspended myoblasts to the culture plate is slower than that of most other cell types present in the cell suspension¹⁰.

C3H mice were used as donors for the serial passage of normal mouse myoblasts. Injuring the muscle by crushing with a strong forceps 2–3 d before culture preparation resulted in an over fivefold increase in the yield of mononucleated myogenic cells obtained from untreated muscle

Fig. 1 *a*, Muscle-forming colonies in a first passage culture of cells obtained from a dystrophic muscle, plated at a density of 4,000 cells per 60-mm plate. Fixed after 13 d. ($\times 10$.) *b*, A section of a muscle-forming colony containing cross-striated multinucleated fibres. Fixed after 18 d in culture. ($\times 308$.)



Fig. 2 A culture of D₁ cells (13th serial passage), containing a network of multinucleated fibres. Fixed 13 d after plating. ($\times 169$.)

($\sim 7 \times 10^6$ cells were obtained from the injured muscles of one thigh).

Mass cultures prepared from normal 2-month-old mouse muscle consisted of mononucleated cells during the first 2 d. A great proportion of them were spindle-shaped, resembling myoblasts obtained from newborn animals. Cell fusion and the formation of a network of multinucleated fibres started on the third or fourth day. In cultures prepared from dystrophic muscle, there was a great excess of fibroblastic cells over myoblasts. Most of these fibroblastic cells apparently stemmed from the connective or fat tissue which occupied a great part of the wasting muscle. Very few short multinucleated fibres were formed.

It was reported previously that outgrowths of mononucleated cells in explants of muscle obtained from dystrophic mice of the same strain failed to form multinucleated fibres: instead, the cells formed 'pseudostraps' consisting of rows of unfused cells'. Similar aggregates of cells were occasionally found in our cultures. In order to test whether the failure of cultures prepared from dystrophic muscle to differentiate was due to a lesion in the myogenic cells, or to the presence of a great excess of non-myogenic cells, cultures were plated at very low densities (500–5,000 per plate). In these conditions, colonies originating from single cells were formed and most of them were composed of non-fusing fibroblastic cells. About 5–10% of the colonies, however, consisted of homogeneous populations of spindle-shaped myoblasts. After several days these colonies contained dense networks of long multinucleated contracting fibres. The proportion of muscle-forming colonies increased markedly in secondary cultures prepared by selective cell passage (Fig. 1*a*, *b*).

The apparently normal morphology of the myotubes obtained in these colonies shows that at least part of the myoblasts obtained from dystrophic muscle of animals homozygous for the *dy* gene are able to proliferate, fuse and differentiate up to the stage of functional, contractile multinucleated myofibres. The poor differentiation of primary cultures prepared from dystrophic muscle thus seems to be caused by the presence of other cell types which diluted the myogenic cells or interfered in some other way with their fusion. It is, however, impossible to conclude from these experiments that in dystrophic muscle all cells belonging to the myogenic lineage are able to differentiate. The possibility that part of the non-fusing clones are abnormal muscle precursor cells cannot be excluded at present.

After establishing the existence of proliferating muscle-forming cells in the primary cultures prepared from dystrophic muscle, attempts were made to propagate these cells. Myogenic colonies obtained from primary cultures

were isolated and serially passaged. In four out of five independent experiments, proliferation decreased and the myogenic cells were lost after 3–4 passages (similar to the results obtained with normal myoblasts¹³). The D₁ line described here originated from a single myogenic colony which was isolated after the third selective passage of a primary culture plated at low density.

The cells of this line (now over 30 passages) are maintained in Dulbecco's modified Eagle's medium supplemented with 20% foetal calf serum. They proliferate as mononucleated cells with a generation time of 24 h. The main phase of cell fusion starts when confluency is reached and in the course of several days a very dense network of multinucleated fibres is formed. Fibre formation is enhanced considerably when medium supplemented with 10% horse serum is used (Fig. 2). Two to three days after the onset of cell fusion, the fibres begin to contract. Contractions are sometimes very vigorous and cause the detachment of the cell layer from the culture plate.

Two lines were established from primary cultures prepared from thigh muscle of 2-month-old normal mice. The line C₁ originated by selective serial passage of myoblasts obtained from thigh muscle 48 h after crush injury and C₂ started from cultures prepared 70 h after injury.

Formation of multinucleated fibres in chick and rat skeletal muscle cultures, is associated with a large increase in creatine kinase activity^{11,12}. The activity of creatine kinase in the D₁ and C₂ lines was tested as a biochemical parameter for differentiation. As can be seen from Table 1, in both lines the activity of creatine kinase in differentiated cultures was over one order of magnitude higher than in cultures collected before cell fusion.

Table 1 Creatine kinase activity in cultures of myogenic cell lines derived from dystrophic mouse (D₁) and normal mouse (C₂)

Cell line	Creatine kinase activity (μ g per mg protein)	
	Mononucleated cells	Differentiated cultures
D ₁	80	2,160
C ₂	123	1,550

Cultures of mononucleated cells (48 h after plating) and differentiated cultures containing multinucleated fibres (day 5) were collected and creatine kinase was assayed as described in Shainberg *et al.*¹¹.

These experiments show that adult dystrophic muscle of mice homozygous for the gene *dy* contain myogenic cells capable of proliferating and forming differentiated myogenic colonies. The high frequency of muscle-forming colonies in the primary cultures plated at low densities indicates that the cells capable of differentiation *in vitro* were not obtained by selection of a very rare back mutant of the *dy* gene. Rather, it shows that the progenitors of the muscle-forming colonies found in the primary cultures constitute a significant part of the mononucleated cell population of the intact dystrophic muscle. Therefore, these cells are most probably homozygous for the *dy* gene.

The ability to obtain large homogeneous cell populations which undergo differentiation in controlled culture conditions should enable comparative studies at the cellular and biochemical levels of the intrinsic properties of myogenic cells obtained from dystrophic and normal muscle. It should be stressed, however, that comparisons of the differentiation of several myogenic cell lines derived from normal rats showed some differences between lines^{13,14}. Therefore, comparative investigations of several lines of dystrophic and normal origin will be necessary in order to distinguish between differences among individual lines acquired during their establishment and the properties related to their

genetic origin. Since the dystrophic lesion might be a disorder expressed only in fully differentiated fibres, it is also necessary for such a study to establish optimal conditions for maximal differentiation and long-term maintenance of the multinucleated fibres formed in cultures.

This investigation was supported by grants from the Muscular Dystrophy Association, Inc., USA, and from the National Institutes of Health.

DAVID YAFFE
ORA SAXEL

Department of Cell Biology,
The Weizmann Institute of Science,
Rehovot, Israel

Received 28 July; accepted 25 October 1977.

- Appenzeller, O. *Arch. Neurol.* **32**, 2–4 (1975).
- Askasas, V., Shapin, S. A. & Milhorat, A. *Arch. Neurol.* **24**, 259–265 (1971).
- Bishop, A., Gallup, B., Sklate, Y. & Dubowitz, V. *J. neur. Sci.* **13**, 333 (1971).
- Parsons, R. *Nature* **251**, 621–622 (1974).
- Gallup, B. & Dubowitz, V. *Nature* **243**, 287–289 (1973).
- Powell, J. A. *Expl Cell Res.* **80**, 251–264 (1973).
- Michelson, A. M., Russell, E. S. & Harman, P. J. *Proc. natn. Acad. Sci. U.S.A.* **41**, 1079–1084 (1955).
- Yasin, R., Van Beers, G., Bulien, D. & Thompson, E. J. *Expl Cell Res.* **102**, 405–408 (1976).
- Yaffe, D. in *Tissue Culture: Methods and Applications* (eds P. F. Kruse & M. K. Patterson) 106–114 (Academic, New York, 1973).
- Yaffe, D. *Proc. natn. Acad. Sci. U.S.A.* **61**, 477–483 (1968).
- Shainberg, A., Yagil, G. & Yaffe, D. *Dev Biol.* **25**, 1–29 (1971).
- Reporter, M. C., Konigsberg, I. R. & Streher, B. L. *Expl Cell Res.* **30**, 410–417 (1963).
- Richler, C. & Yaffe, D. *Dev Biol.* **23**, 1–22 (1970).
- Yaffe, D. & Saxel, O. *Differentiation* **7**, 159–166 (1977).

Influence of the main histocompatibility complex on ageing in mice

CONSIDERATION of the facts of mammalian cell 'transformation' by viruses led us to the proposition that only a few genes or gene systems need necessarily be involved in the ageing process^{1,2}. These views accord in principle with conclusions derived from more recent analyses of the genetic basis for the increase in lifespan of hominid species in the last several hundred thousand years, namely that mutations at no more than about 0.6% of the total genome could be responsible for the increase^{3,4}. We describe here a study of ultimate lifespans (as reflected by tenth deciles of survivorship) in congenic mice which suggests that the main histocompatibility complex (MHC) is one of the gene systems involved in the control of ageing.

All vertebrate species so far investigated—birds and amphibia as well as mammals—possess an MHC: for example, the *H-2* system in the mouse and the HLA system in man. Each of these occupies a short chromosomal region of sufficient size to accommodate several hundred gene loci, although only 6–12 are as yet identified in any one species. The MHC represents a so-called 'super-gene' system, a term referring to a cluster of genes influencing the same category of functions. It influences or regulates at least the following functions⁵: the recognition phase of cellular immunity as manifested in the mixed lymphocyte reaction; cell-mediated lymphocytotoxicity; the development of specific suppressor cells important for immunorecognition; susceptibility to a number of viruses; susceptibility to the development of various autoimmune diseases; the ability to mount an immune response to certain specific antigens (via the immune response genes); the age-specific maturation rates, peaks and rates of decline of different immune response capacities; components of the complement system; T/B-cell collaboration (helper cells); the quantitative expression of the Thy-1 or 'theta' antigen; and levels of plasma testosterone and testosterone-binding protein. In addition gene products at the D and K loci of the *H-2* system may be required for recognition of 'self'^{6,7}.

Ageing is associated with marked alterations in immune response capacity, particularly including *H-2* associated

Table 1 Tenth decile of survivorship and mean lifespan in male mice congenic at the H-2 chromosomal region and on three different backgrounds

Background Strain	C57BL/10 H-2 allele	1	2	3	10th decile (weeks)	4	5	6	7	1	2	Mean survival (weeks)	3	4	5	6	7
1 B10.AKM	<i>m</i>	139±3.7								99±3.2							
2 B10.Br/Sg	<i>u</i>	x	149±1.1							xx	113±3.8						
3 B10.PL	<i>k</i>	x		153±1.7						xx	x	125±3.1					
4 B10.A/Sg	<i>a</i>	x	xx		154±0.4					xx	x		128±4.1				
5 B10.D2/n	<i>d</i>	x	xx			155±0.5				xx				122±4.0			
6 C57BL/10	<i>b</i>	x	xx				155±0.4	170±0.8		xx	xx	x			x	134±3.0	
7 B10.RIII	<i>r</i>	xx	xx	xx	xx	xx				xx	xx	xx	x		xx		141±3.4
A																	
8 A.BY	<i>b</i>	114±3.5								85±2							
9 A.SW	<i>s</i>		123±6.6								90±2.1						
10 A.CA	<i>f</i>	xx		127±3.0								85±2.8					
11 A/Wy	<i>a</i>	xx	x	xx	134±1.7					xx		xx	97±2.5				
C3H																	
12 C3H/HeDi	<i>k</i>	138±0.7								98±3.0							
13 C3H.JK	<i>j</i>	xx	146±1.4							xx	112±2.8						
14 C3H.SW	<i>b</i>	xx	xx	150±1.2						x		108±3.7					

x, Indicates significant difference at the 0.05 and xx at the 0.02 probability levels.

functions, and probably also in mechanisms for distinguishing 'self' from 'non-self'. These alterations might represent primary pathogenetic processes at least in part directly responsible for ageing, or they might constitute largely secondary phenomena⁸. That the MHC might be fundamentally involved in ageing has been suggested on strictly immunological grounds by several workers^{8,9,10}. Indeed, its involvement is a necessary corollary of the immunological theory of ageing⁸.

Direct evidence for an effect of MHC on ageing might be gained from observation of the survival patterns of congenic mice. These mice are prepared by selective breeding on any particular strain background; for example on C57BL, C3H or A strain backgrounds. Within each background, strains of mice congenic at H-2 are considered genetically 'identical' except for the relatively short chromosomal region carrying the H-2 system. In this study, therefore, we compared lifespans for both sexes of seven strains of mice congenic for H-2 on a C57BL/10 background, four on an A background, and three on a C3H background.

The 14 strains are listed in Tables 1 and 2 according to background and H-2 alleles. They were obtained either as 6-8-week-old animals, or as breeding pairs, from the Jackson Laboratory (courtesy of Dr George Snell). About 60 animals of each sex and strain were set up at two months of age, housed six per cage, and maintained on standard Purina laboratory chow and water *ad libitum*. The cages were inspected daily. The animals were allowed to live out their natural lifespans. They were not regrouped following deaths of any in the cages. The lifespan of each animal was calculated to the nearest week. Mean lifespan and standard error were determined for both sexes of each group for both the total group and for the last tenth of

survivors of a group, the so-called 'tenth decile'.

Tables 1 and 2 show the tenth decile of survivorship and the mean age of death of male and female mice for all strains. For both sexes the survival of the strains congenic upon the C57BL/10 background tended to exceed the survival of the other two background sets. This variation probably reflects the operation of some of the many non-H-2 genetic differences between the three background sets; however, we are here primarily concerned with differences within each set, as that would represent an H-2 influence. It is clear that within each set there were frequent and striking differences in strain lifespan as judged both by tenth decile survivorship and mean ages at death. For example, on the C57BL/10 background the strain B10.AKM male mouse had a significantly shorter survival than all six partner strains, and B10.AKM females shorter than five of six partner strains.

We emphasise that the mean age of death of the whole population is in fact a poor criterion of differences of physiological ageing rates between strains of mice, for it is highly susceptible to environmental influences and specific disease patterns. The ages of death of the longest-lived individual survivors in infinite populations provided in fact the best single comparative criterion of physiological ageing, as cogently argued by Sacher¹¹, for it is in fact relatively non-susceptible to influence by environmental factors or disease processes including tumour incidence. Lacking sufficiently large or infinite populations, the mean ages at death of the tenth deciles of survivorship affords a reasonably accurate estimate for comparison and possesses similar advantages¹². By this criterion as well as that of mean age at death, we noted very considerable variations among lifespans of congenic lines (Tables 1, 2). These were comparable in degree to the variations observed among

Table 2 Tenth decile of survivorship and mean lifespan in female mice congenic at the H-2 chromosomal region and on three different backgrounds

Background Strain	C57BL/10 H-2 allele	1	2	3	10th decile (weeks)	4	5	6	7	1	2	Mean survival (weeks)	3	4	5	6	7
1 B10.AKM	<i>m</i>	143±2.3								101±3.2							
2 B10.PL	<i>u</i>		145±1.7							xx	111±4.0						
3 C57BL/10	<i>b</i>	x		148±1.2						xx		120±3.1					
4 B10.D2/n	<i>d</i>	xx	xx	xx	154±0.8					x			116±5.0				
5 B10.BR/Sg	<i>k</i>	xx	xx	xx		161±2.1				xx	xx			125±3.5			
6 B10.A/Sg	<i>a</i>	xx	xx	xx	xx		164±2.9			xx	x				122±3.9		
7 B10.RIII	<i>r</i>	xx	xx	xx	xx	xx		165±1.0		xx	xx		x				129±3.9
A																	
8 A.BY	<i>b</i>	128±2.3								84±2.6							
9 A.SW	<i>s</i>		130±4.3							x	89±2.8						
10 A.CA	<i>f</i>			134±3.4						x		89±3.5					
11 A/Wy	<i>a</i>	xx			138±2.4					xx			94±2.9				
C3H																	
12 C3H/HeDi	<i>k</i>	133±2.5								94±2.8							
13 C3H.JK	<i>j</i>		136±1.1							xx	105±2.6						
14 C3H.SW	<i>b</i>	xx	xx	151±1.4						xx		109±3.8					

x, Indicates significant difference at the 0.05 and xx at the 0.02 probability levels.

unrelated inbred strains of mice¹². One might have expected a much greater uniformity of lifespans within each congenic set than actually observed unless the *H-2* region itself exerts a significant effect on ageing.

Since the MHC represents the master genetic control or regulatory system for immune function, particularly thymus-dependent function, which declines markedly with age, we suggest that the lifespan differences noted may reflect a complex age-related effect of the MHC on immune mechanisms. Evidence supporting this interpretation comes from separately reported studies of immune function in nine of these same strains¹³ in which the response to different mitogens in mice from 2 to over 30 months of age were compared. On the C57BL/10 background the longest-lived strains, B10.RIII, displayed the highest response to phytohaemagglutinin throughout most of life. The shortest-lived strain, B10.AKM, displayed the lowest response. Survival patterns of the A strain mice also accorded the age-specific phytohaemagglutinin responses. Shorter lifespan could on the whole be correlated with an earlier decline in immune response capacity, as judged by these and other criteria¹³.

It is also possible, as suggested by Bodmer¹⁴, that a number of genes exist in the MHC region other than those determining the already recognised *H-2* antigens, and that these additional genes control the synthesis of differentiation antigens or a class of recognisers. If such be the case, an alternate or additional explanation for the observed effect of the MHC on ageing might be postulated. With regard to a possible role of the MHC in human ageing, the genetic heterogeneity of man renders the collection of useful data difficult. It has recently been noted by Yunis and Greenberg¹⁸, however, that the frequency of HLA-B8 is decreased in old cohorts of human females, suggesting the possibility of an increased mortality rate in females carrying this marker.

Our study represents one of several necessary steps in dissecting the complex genetic parameters of ageing, which are undoubtedly polygenic but are also likely to be an analysable finite number. Additional experiments, including genetic marker (MHC) identification in *F*₁ and back-cross studies between appropriate congenic strains, will be required to verify the apparent association between longevity and *H-2* type within any congenic set. Nevertheless, our existing data from 14 strains of mice divided into three sets congenic on three different backgrounds do strongly suggest that the MHC may be one of the principle genetic systems involved in controlling lifespan. This suggestion is in line with suggestions of Walford^{1,2}, Cutler³ and Sacher⁴, that ageing rates may reflect the operation of a finite number of gene systems, and not necessarily of the whole genome. The MHC may well be one of these systems.

We thank Ken Morris and Melvinia Harris for technical assistance and Kay Cheney for statistical assistance. This work was supported by USPHS research grants CA-12788 and AG-00424. Computing assistance was obtained from Health Sciences Computing Facility, UCLA, sponsored by Public Health Service grant RR-3.

GEORGE S. SMITH
ROY L. WALFORD

Department of Pathology,
School of Medicine,
University of California,
Los Angeles, California 90024

Received 21 July; accepted 24 July 1977.

1. Walford, R. L. *Gerontologia* 18, 243-246 (1972).
2. Walford, R. L. *Fed. Proc.* 33, 2020-2027 (1974).
3. Cutler, R. G. *Proc. natn. Acad. Sci. U.S.A.* 72, 4664-4668 (1975).
4. Sacher, G. A. in *Antecedents of Man and After. Primates: Primates Functional Morphology and Evolution* (ed. Tuttle, R.) 417-441 (Mouton, The Hague, 1975).
5. Walford, R. L. in *The HLA System, New Aspects* (ed. Ferrara, G. B.) 105-127 (Elsevier/North Holland, Amsterdam, 1977).
6. Bevan, M. J. *Nature* 256, 419-421 (1975).

7. Doherty, P. C. & Zinkernagel, R. M. *Lancet* i, 1406-1408 (1975).
8. Walford, R. L. *The Immunologic Theory of Aging* (Munksgaard, Copenhagen, 1969).
9. Walford, R. L. *Lancet* ii, 1226-1229 (1970).
10. Yunis, E. J., Fernandes, G. & Greenberg, W. J. in *Immunodeficiency Workshop: Birth Defects, Original Series* 11, 85-95 (Plenum, New York, 1973).
11. Sacher, G. A. in *The Lifespan of Animals, CIBA Fnd Coll. on Ageing* 5, 115-151 (Little Brown, Boston, 1959).
12. Smith, G. S., Walford, R. L. & Mickey, M. R. *J. natn. Cancer Inst.* 50, 1195-1213 (1973).
13. Meredith, P. & Walford, R. L. *Immunogenetics* (in the press).
14. Bodmer, W. F. *Nature* 237, 139-145 (1972).
15. Yunis, E. J. & Greenberg, L. J. in *Genetic Effects on Aging* (ed. Harrison, D.) (National Foundation/March of Dimes, New York, in the press).

Possibility of EB virus preferentially transforming a subpopulation of human B lymphocytes

PERMANENT lymphoblastoid cell lines can be established by 'transformation' of human lymphocytes with EB virus^{1,2}. The lines secrete immunoglobulin (Ig) *in vitro*^{3,4} and represent, morphologically, an intermediate stage between the resting lymphocyte and the fully developed plasma cell^{5,6}. It has been suggested that the EB virus stimulates inactive B lymphocytes to secrete immunoglobulin and that the virus is behaving, in this respect, like a polyclonal B cell mitogen. The evidence comes from observations that most (possibly all) human B lymphocytes carry surface receptors for EB virus⁸, that an increase in Ig secretion is detectable within a few days of adding the virus to a culture of lymphocytes⁷, and that in the early stages of growth, lymphoblastoid lines established in this way secrete multiple classes of Ig heavy and light chain^{4,9,10}. It seems, however, that only a fraction of 1% of lymphocytes in an EB virus-infected culture respond either by Ig synthesis or by proliferation and furthermore that the two events occur simultaneously^{7,11,12}. The possibility therefore remains that EB virus does not stimulate Ig synthesis *de novo* but selectively 'transforms' (that is, induces to proliferate) that minor population of B cells which has already begun to secrete Ig. This alternative is supported by our analysis of the major classes of Ig heavy and light chains secreted by lymphoblastoid cell lines (derived from peripheral blood lymphocytes) in relation to the age and clinical status of the donors.

The data are set out in Table 1. The most striking finding is that, of fifty unselected lines derived from cord bloods, forty-nine secrete only IgM. This is at variance with the expected outcome of polyclonal stimulation of resting B cells, since the ability to synthesise cytoplasmic Ig of differing heavy chain classes in response to a polyclonal B cell mitogen seems to be acquired earlier in ontogeny than the class and subclass segregation of cell surface immunoglobulin determinants that is already well established among the circulating lymphocytes of the human foetus many weeks before birth^{13,14}. Although IgM-secreting cells have been shown to predominate among pokeweed-stimulated human cord lymphocytes, IgG secretors form a substantial proportion in most samples and, in some cases at least, IgA-producing cells are also readily found¹⁵. It therefore seems incompatible with this mode of action of EB virus that no γ or α heavy chains could be detected in the supernatants of any of our cord blood lines even when tested repeatedly during the early weeks following their establishment *in vitro* and even though polyclonal composition could still be demonstrated by the presence of both kappa and lambda light chains. Conversely, our findings are in keeping with the view that EB virus induces proliferation in those rare cells which are actively secreting immunoglobulin since, for the first few weeks after birth, the healthy human infant synthesises Ig exclusively of class M (ref. 16).

Adult blood lymphocytes stimulated with pokeweed mitogen typically contain populations of B cells secreting IgM, IgG and IgA respectively, in the approximate proportions of 2:2:1 (refs 15 and 17). By comparison, IgM secretors seem to be relatively over-represented among the monoclonal lines derived from healthy adults and under-represented among those from infectious mononucleosis patients (Table 1). The difference between the two groups is difficult to explain if the lines are derived from resting blood B cells, but since infectious mononucleosis is characterised

Table 1 Patterns of Ig secretion by human lymphoblastoid cell lines

Source of blood Lymphocytes*	No. of cell lines (no. of different donors)	Ig secreted†						
		G	Heavy chain		None‡	Light chain		
			A	M		K	L	None
Cords	Total 50(25)							
	Monoclonal Ig secretors 39(20)	0	0	49	1	30	29	1
		0	0	39	—	20	19	—
Adults (no recent infection)§	Total 79(62)							
	Monoclonal Ig secretors 66(59)	31	10	49	0	54	40	0
		20	8	38	0	39	27	0
Adults (infectious mononucleosis)	Total 19(16)							
	Monoclonal Ig secretors 17(15)	9	5	7	0	14	7	0
		7	5	5	0	12	5	0

*All cord blood lines, all infectious mononucleosis-derived lines and all but two of the lines from other adult donors were established in our laboratory. †Ig secretion was detected by a highly sensitive and reproducible haemagglutination-inhibition technique⁴. In most cases supernatants had been tested on several occasions.

‡One cord-derived line (FAL₁) has consistently failed to secrete any detectable Ig (ref. 4). Morphologically it is indistinguishable from other lymphoblastoid lines, and complete EB virus particles were seen in electron microscope preparations. The cells have surface Ig MK determinants (by indirect immunofluorescence) and react strongly with a rabbit antiserum specific for human B lymphoid cells.

§Although described as 'adults', three of these donors were below the age of 16 yr. The group comprised healthy individuals and those suffering from genetic (including cytogenetic) disorders or from malignant disease not affecting the lymphoreticular system.

¶Lines were considered monoclonal when only a single class of Ig heavy chain and single class of light chain could be detected in the supernatant.

||The difference between these two groups in the proportion of monoclonal lines secreting M heavy chain is significant ($\chi^2 = 4.3$; $P < 0.05$). There are no significant differences between any group in the proportion of lines secreting K or L light chains.

by a substantial increase in serum Ig of all three major classes¹⁸, the population of Ig-secreting cells in the circulation is likely to be markedly disturbed. If this is the population which gives rise to lymphoblastoid lines, a deviation from the normal distribution of G, M and A secretors would not be unexpected. It would also provide a rational explanation for the observation that lymphoblastoid lines can be established exceptionally easily from the blood of patients in the acute or early convalescent phase of infectious mononucleosis¹⁹⁻²¹ and other virus infections^{22,23}, when the absolute number of Ig-secreting cells in the circulation is abnormally high.

Arguments based on the study of Ig secretion by monoclonal lymphoblastoid lines would, of course, be invalid if there was any suspicion of a selective advantage for the growth of cells producing Ig of a particular class, or if the commitment of a given cell and its progeny to secrete a particular class of heavy chain were unstable in the conditions of culture. On the first point, our experience has been that when multiple aliquots of 10-100 cells are taken, at an early stage of growth, from a line which is secreting comparable amounts of two immunoglobulin molecules (distinguished by heavy and/or light chain class), the subcultures ultimately give rise to approximately equal numbers of clones secreting each of the parental Ig species. Selection of the dominant clone from a newly established line therefore seems to be a random process with respect to class of Ig secreted. On the second point, in spite of one contradictory report²⁴, it seems that lymphoblastoid cell clones secreting more than one class of heavy and/or light chain are exceptionally rare^{4,25}, while stability of the commitment to secrete a particular class of Ig is one of the most striking features of human lymphoblastoid lines²⁶. We have been unable to detect any change in the pattern of Ig synthesis among several hundred clones derived from lines exposed to a variety of physical and chemical mutagens, although a considerable number of other biochemical changes have been induced^{27,28}.

The distribution of monoclonal heavy and light chains ultimately produced by lines from a particular set of donors is therefore likely to be a valid index of the relative proportions of their blood lymphocytes secreting (or committed to secrete) each class of Ig and susceptible to EB virus 'transformation'.

It is important to an understanding of the mechanism of EB virus transformation to know whether the stage of differentiation of the host B lymphocyte is a critical factor. Our conclusion, that

there is preferential, if not exclusive, transformation of those cells already secreting Ig *in vivo* (though mature plasma cells are evidently insusceptible²⁵), seems to be more compatible with the available data than the proposition that EB virus acts as a polyclonal B cell mitogen. If the subpopulation of Ig-secreting blood lymphocytes can be isolated—for example, by velocity gradient sedimentation²⁹—the question may be resolved.

We thank Mrs Marilyn Woodward, Mrs June Jenkins, Mr Gordon Martin and Mr Ross Maynard for assistance, and Mr J. Davies for advice on statistical analysis.

C. M. STEEL
JUDITH PHILIPSON
ELIZABETH ARTHUR
SUSAN E. GARDINER
MARJORIE S. NEWTON
R. V. MCINTOSH

MRC Clinical and Population Cytogenetics Unit,
Western General Hospital, Crewe Road,
Edinburgh, UK

Received 26 July; accepted 30 October 1977.

- Diehl, V., Henle, G., Henle, W. & Kohn, G. in *Haemic Cells In Vitro* (ed. Farnes, P.) 92 (Williams and Wilkins, Baltimore, 1969).
- Nilsson, K., Klein, G., Henle, W. & Henle, G. *Int. J. Cancer* **8**, 443-450 (1971).
- Fahey, J. L., Finegold, I., Rabson, A. S. & Manaker, R. A. *Science* **152**, 1259-1261 (1966).
- Evans, J., Steel, C. M. & Arthur, E. *Cell* **3**, 153-158 (1974).
- De Harven, E. *Cancer Res.* **27**, 2447-2464 (1967).
- Chandra, S., Moore, G. E. & Brandt, P. M. *Cancer Res.* **28**, 1982-1989 (1968).
- Rosen, A., Gergely, P., Jondal, M., Klein, G. & Britton, S. *Nature* **267**, 52-54 (1977).
- Jondal, M. & Klein, G. *J. exp. Med.* **138**, 1365-1378 (1978).
- Rechet, J. M., Nilsson, K. & Klein, G. in *Oncogenesis and Herpes Viruses* (ed Briggs, P. M., de The, G. & Payne, L. M.) (Int. Agency Res. Cancer, Lyon, 249 1972).
- Glade, P. R. & Papageorgiou, P. S. *In Vitro* **9**, 202-215 (1973).
- Robinson, J. & Miller, G. J. *Viral* **15**, 1065-1072 (1972).
- Thorley-Lawson, D. A., Chess, L. & Strominger, J. L. *J. exp. Med.* **146**, 459-508 (1977).
- Kearney, J. F. & Lawton, A. R. *J. Immun.* **115**, 677-681 (1975).
- Lawton, A. R., Kinkade, P. W. & Cooper, M. D. *Fedn Proc.* **34**, 33-39 (1974).
- Wu, L. Y. F., Blanco, A., Cooper, M. D. & Lawton, A. R. *Clin. Immun. Immunopath.* **5**, 208-217 (1976).
- Lawton, A. R., Self, K. S., Royal, S. A. & Cooper, M. D. *Clin. Immun. Immunopath.* **1**, 84-93 (1972).
- Janossy, G. & Greaves, M. *Transplant. Rev.* **24**, 177-236 (1975).
- Sutton, R. N. P., Reynolds, K., Almond, J. P., Marston, S. D. & Edmond, R. T. D. *Clin. exp. Immun.* **13**, 359 (1973).
- Poppe, J. H. *Nature* **216**, 810-811 (1967).
- Henle, G., Henle, W. & Diehl, V. *Proc. natn. Acad. Sci. U.S.A.* **59**, 94 (1968).
- Steel, C. M. & Edmond, E. *J. natn. Cancer Inst.* **47**, 1193-1201 (1971).
- Glade, P. R., Paltrowitz, I. M. & Hirschhorn, K. *Bull N.Y. Acad. Med.* **45**, 648-650 (1969).

23. Stevens, D. P., Barker, L. F., Fike, R., Hopps, H. E. & Meyer, H. M. *Proc. Soc. exp. Biol. Med.* **132**, 1042-1046 (1969).
 24. Bloom, A. D., Choi, K. W. & Lamb, B. J. *Science* **172**, 382-383 (1972).
 25. Nilsson, K. *Int. J. Cancer* **8**, 432-442 (1971).
 26. Nilsson, K. *Hemat. Blood Transfus.* **20**, 253-264 (1977).
 27. Povey, S. *et al. Ann. Hum. Genet.* **36**, 247-266 (1973).
 28. Arthur, M. F. *et al. Ann. Hum. Genet.* **39**, 33-42 (1975).
 29. Andersson, J., Laffeur, L. & Melchers, F. *Eur. J. Immun.* **4**, 170-180 (1974).

Murine mesenteric and peripheral lymph nodes: a common pool of small T cells

GRISCELLI *et al.* have demonstrated¹ the existence of two populations of lymphoblasts with distinct migratory characteristics in the rat. One population was obtained from the mesenteric lymph nodes which migrate to the lamina propria of the small intestine and the other from peripheral lymph nodes which failed to migrate to the gut but were found in the spleen. These observations have been confirmed subsequently in the mouse²⁻⁵ and in the sheep⁶. In mice the different migratory patterns of lymphoblasts of mesenteric or peripheral origin were shown to operate at the level of the T lymphoblast⁷. We have investigated whether this dichotomy extends to the migration of ⁵¹Cr-labelled small T lymphocytes and have found that less than 2% of the injected small T lymphocytes localise in the small intestine (excluding Peyer's patches) and that mesenteric and peripheral small T cells migrate equally well through mesenteric, peripheral lymph nodes and Peyer's patches. There is a slight but consistent tendency for mesenteric T cells to localise more in the liver and less in the spleen than peripheral T cells. We conclude that the distinctive migration which characterises lymphoblasts in mice does not apply to small lymphocytes.

Six- to twelve-week-old mice of the inbred strains C₃H/AnF (Cumberland View Farms, Tennessee) and CBA (Department of Bacteriology and Immunology, Glasgow) matched in each experiment for age and sex were used. Cell suspensions from mesenteric or peripheral (axillary, brachial and inguinal) lymph nodes were prepared by conventional methods⁷ and T lymphocytes separated by passage through nylon wool columns as described by Julius *et al.*⁸. The cells in the eluate (>94% thy 1⁺) were labelled with (⁵¹Cr) sodium chromate⁷ and their distribution in syngeneic recipients followed at intervals (1, 3 and 24 h) after intravenous transfer (Table 1). Little radioactivity was recovered from the small intestine, regardless of the source of the cell

inoculum, at 1, 3 (not shown) or 24 h after transfer. Mesenteric T cells (MLN-T) showed a slightly increased localisation in the Peyer's patches (at 1 h) and small intestine (at 1 and 24 h) when compared with peripheral T cells (PLN-T) but this has not been a consistently significant finding (see experiment 2 in Table 1). There was also an enhanced accumulation of MLN-T cells in the liver, which was sometimes accompanied by a decreased localisation of the MLN-T cells in the spleen and lymph nodes of the recipients. Variable percentages of damaged cells could not account for the observed differences in liver localisation, since the viability of both MLN-T and PLN-T cell suspensions before injection was greater than 97%.

In the next series of experiments we attempted to separate recirculating T cells from non-recirculating components and to enhance the differences between the migratory patterns of MLN or PLN cells by passage through syngeneic intermediary hosts. For this purpose ⁵¹Cr-labelled MLN and PLN lymphocytes were injected into primary recipients which were killed 24 h later. Cells from the mesenteric or peripheral lymph nodes were then collected and the migration of the labelled cells localised in these lymph nodes was followed after intravenous transfer into secondary recipients. Since it has been shown that only a very small minority of ⁵¹Cr-labelled B cells localise in the lymph nodes by 24 h (ref. 7), the population of labelled cells obtained from the lymph nodes of the intermediate recipients should consist of more than 90% T lymphocytes. The results obtained (Table 2) are in agreement with our previous experiments (Table 1). MLN cells had an increased tendency to accumulate in the liver and a decreased localisation in the spleen, compared with PLN cells. There were no differences, however, in the distribution of both MLN and PLN cells into the small intestine or Peyer's patches.

We have found that the mesenteric lymph nodes of "unprimed" mice contain consistently more pyroninophilic blast cells and incorporate more ¹²⁵IUdR than peripheral lymph nodes and that these cells were not removed by a nylon wool column (Table 3). Therefore, these cells could account for some of the differences observed in the migration of ⁵¹Cr-labelled MLN-T and PLN-T cells. To lessen the contribution made by lymphoblasts to the migratory patterns of MLN-T and PLN-T cells these cells were depleted of lymphoblasts by velocity sedimentation through a continuous gradient of 5-20% Ficoll⁹. After Ficoll separation the percentage of pyroninophilic cells in both

Table 1 Localisation of ⁵¹Cr-labelled mesenteric T cells (MLN-T) and peripheral T cells (PLN-T) 1 and 24 h after i.v. transfer into syngeneic recipients

Experiment	Donor cells	Time (h)	Localisation in*						
			PLN	MLN	Spleen	Liver	Lung	PP	SI
1	PLN-T	1	3.8±0.6	8.7±1.7	31.1±3.8	8.5±0.4	6.2±0.7	2.9±0.1	1.1±0.2
	MLN-T	1	3.9±0.1	9.3±0.4	28.0±4.7	12.2±1.2†	7.6±0.2†	3.9±0.03§	1.6±0.3†
1	PLN-T	24	6.7±0.3	15.3±2.2	20.6±2.2	7.9±0.7	2.3±0.4	2.1±0.5	0.9±0.2
	MLN-T	24	6.3±0.5	17.1±1.5	22.4±2.8	12.3±1.7†	2.3±0.4	2.6±0.4	1.2±0.1†
2	PLN-T	24	4.1±0.8	10.9±0.6	18.9±0.8	5.7±0.4	1.8±0.4	1.6±0.3	0.9±0.5
	MLN-T	24	3.7±0.9	8.5±0.2§	16.7±1.0	11.2±0.2§	1.2±0.2	1.6±0.2	1.2±0.1

PP, Peyer's patches; SI, small intestine.

Nylon wool separated mesenteric and peripheral T cells were labelled *in vitro* with ⁵¹Cr (sodium chromate, NEN, Chicago or Radiochemical Centre, Amersham) at a concentration of 50 µCi per 10⁸ cells per ml for 30 min at 37 °C (ref. 7). The cells were washed three times and passed quickly through a packed column of glass wool saturated in 10% FCS containing media to remove damaged or dead cells. The viability of the cell suspensions was then assessed with the help of the Trypan blue exclusion test (0.1%) and 10⁷ viable cells in approximately 0.2 ml injected into the lateral tail vein of syngeneic recipients. The animals were killed 1 and 24 h after cell transfer and the radioactive content of various organs was measured in a Gamma Spectrometer (Packard, USA).

*Results are expressed as a percentage of the injected radioactive dose. Each value represents the mean (± s.d.) of 4-6 mice (C₃HAn/F for experiment 1 and CBA for experiment 2). Statistical analysis was by Student's *t* test.

†*P* < 0.05.

‡*P* < 0.01.

§*P* < 0.001.

Table 2 Secondary migration of ^{51}Cr -labelled mesenteric cells (MLN) and peripheral cells (PLN) after selection in intermediate hosts

Donor cells	Time (h)	Localisation in*						
		PLN	MLN	Spleen	Liver	Lung	PP	SI
PLN	1	2.7 $\pm$ 0.4	12.2 $\pm$ 2.3	38.8 $\pm$ 3.8	7.6 $\pm$ 1.1	4.5 $\pm$ 1.0	4.1 $\pm$ 0.3	1.7 $\pm$ 0.9
MLN	1	2.7 $\pm$ 0.2	14.9 $\pm$ 1.5†	31.2 $\pm$ 2.2†	9.2 $\pm$ 0.8†	4.1 $\pm$ 0.7	4.3 $\pm$ 0.9	1.2 $\pm$ 0.2
PLN	24	3.5 $\pm$ 0.5	18.7 $\pm$ 2.8	18.7 $\pm$ 1.4	6.4 $\pm$ 0.6	2.9 $\pm$ 1.3	3.3 $\pm$ 0.3	1.6 $\pm$ 0.6
MLN	24	3.5 $\pm$ 0.8	17.0 $\pm$ 2.1	16.5 $\pm$ 1.4†	8.1 $\pm$ 0.9†	2.2 $\pm$ 0.6	3.4 $\pm$ 0.4	2.1 $\pm$ 0.8

Peripheral and mesenteric lymph node cell suspensions were labelled with ^{51}Cr and depleted of damaged cells as described in legend to Table 1. Approximately 2×10^7 – 3×10^7 viable cells were injected intravenously into syngeneic recipients (primary hosts). The animals were killed 24 h later and cells were collected from either peripheral or mesenteric lymph nodes. Cell suspensions were rapidly passed through glass wool columns to remove dead or damaged cells, and after this process cell viability was greater than 98% for both cell suspensions. 10^7 cells were injected into the tail vein of syngeneic recipients (secondary recipients). These animals were killed 1 and 24 h after cell transfer and the radioactive content of various organs was measured in a Gamma Spectrometer.

*Results are expressed as a percentage of the injected radioactive dose ($\sim 1 \times 10^4$ – 3×10^4 c.p.m.). Each value represents the mean ($\pm$ s.d.) of 3–6 $\text{C}_3\text{H}/\text{AnF}$ mice. For statistical analysis see legend to Table 1.

MLN-T and PLN-T suspensions was less than 1%. Removal of lymphoblasts abolished almost completely any differences in the distribution of the two cell types (Table 4). There was still—but only 24 h after transfer—an increased accumulation in the liver and a decreased localisation in the spleen of the MLN-T cells. Note that the viability of both cell suspensions studied was superior to 97%.

than a species difference. It may be that as ^3H -uridine-labelled mesenteric cells³ and ^{51}Cr -labelled TDL (ref. 11) migrate in high numbers to Peyer's patches in the mouse, these structures may account for much of the gut-localised ^{51}Cr -labelled cells which were found in the sheep, since Peyer's patches were not removed¹⁰. It must be also noted that in the sheep studies¹⁰ no attempt was made to separate large and small lymphocytes. In our investigation this

Table 3 Size distribution and ^{125}I -UdR uptake of cells in MLN and PLN cell suspensions

	Percentage cell type*			^{125}I -UdR uptake†
	Pyrinophilic lymphoblast	Medium	Small	
Unseparated PLN	2.1	5.8	92.0	2,432 $\pm$ 75
Separated PLN	1.9	6.9	92.0	2,557 $\pm$ 81
Unseparated MLN	4.7	9.2	86.0	4,019 $\pm$ 86
Separated MLN	5.5	8.1	86.0	4,259 $\pm$ 64

*Cytocentrifuge preparations of unseparated or nylon wool column separated MLN and PLN were prepared and stained with methyl green pyronin. Differential counts were performed on 500 cells under $\times 100$ magnification.

†Nylon-wool separated MLN-T or PLN-T cells were incubated *in vitro* with iododeoxyuridine- (^{125}I) (N.E.N., Chicago) at a concentration of 0.5 μCi per 10^7 cells per ml for 60 min at 37 °C. The cells were washed extensively and the amount of radioactivity incorporated measured in a Gamma Spectrometer. Results are expressed as c.p.m. per 10^7 cells. Each value represents the mean $\pm$ s.d. of six replicates ($\text{C}_3\text{H}/\text{AnF}$ mice).

We cannot therefore find a subpopulation of small mesenteric T cells which selectively migrates to the small intestine as happens with mesenteric lymphoblasts. Our results contrast with findings obtained in the sheep¹⁰, where large numbers of ^{51}Cr -labelled T cells obtained from the intestinal lymphatics were found to migrate to the small intestine, but that T cells from the lymph draining the peripheral lymph nodes did not. There could be several reasons for these differences between mice and sheep, other

separation partially abolished the small migratory differences observed between MLN-T and PLN-T cells. Since all studies using rodents, however, have used lymph node cell suspensions, whereas those in the sheep involve the use of cells drained from the lymphatics, it is conceivable that there exists in rodents a population of T cells which recirculates exclusively through the small intestine directly into the intestinal and thoracic duct lymph, without passing through the mesenteric lymph nodes. If this is the case,

Table 4 Localisation of lymphoblast-depleted ^{51}Cr -labelled mesenteric (MLN-T) and peripheral (PLN-T) T lymphocytes 1 and 24 h after transfer into syngeneic recipients

Donor cells	Time (h)	Localisation in*						
		PLN	MLN	Spleen	Liver	Lung	PP	SI
PLN-T	1	2.2 $\pm$ 0.4	9.9 $\pm$ 1.2	36.9 $\pm$ 2.7	10.0 $\pm$ 1.5	10.3 $\pm$ 1.0	2.6 $\pm$ 0.5	1.4 $\pm$ 0.4
MLN-T	1	2.5 $\pm$ 0.7	8.9 $\pm$ 1.0	35.3 $\pm$ 1.2	10.7 $\pm$ 0.8	9.3 $\pm$ 1.0	2.4 $\pm$ 0.5	1.5 $\pm$ 0.6
PLN-T	24	3.7 $\pm$ 0.6	16.7 $\pm$ 1.3	22.2 $\pm$ 1.7	10.4 $\pm$ 1.4	2.0 $\pm$ 0.8	2.2 $\pm$ 0.3	1.4 $\pm$ 0.9
MLN-T	24	3.3 $\pm$ 0.5	16.2 $\pm$ 1.9	18.6 $\pm$ 1.0†	12.8 $\pm$ 1.0†	1.9 $\pm$ 0.4	2.4 $\pm$ 0.4	1.3 $\pm$ 0.2

Mesenteric and peripheral T cells were first obtained by nylon wool column separation. Cells were labelled with ^{51}Cr as described and depleted of any damaged cells by passage through glass wool. Approximately 5×10^7 cells were then layered on top of a 30-ml continuous gradient of 5–20% Ficoll (in PBS supplemented with 1% FCS)⁹. The gradients were centrifuged at 70g for 30 min at 4 °C. The bottom 12 ml were discarded and the remaining cells were washed three times in a large volume of PBS. After separation, 97–98% of the cells were viable as determined by exclusion of 0.1% Trypan blue. Approximately 5×10^6 to 10^7 cells were injected intravenously into syngeneic recipients which were killed 1 and 24 h later. The radioactive content of various organs was measured in a Gamma Spectrometer.

*Results are expressed as a percentage of the injected radioactivity. Each value represents the mean ($\pm$ s.d.) of 4–5 $\text{C}_3\text{H}/\text{AnF}$ mice. For statistical analysis see legend to Table 1.

then this population would be detected in the intestinal lymph but not in the mesenteric lymph nodes. This explanation is, however, unlikely for two reasons: Sprent¹¹ found that only 2.5% of ⁵¹Cr-labelled murine thoracic duct lymphocytes migrate to the small intestine (allowing for localisation in Peyer's patches, see Table 6 in ref. 11) and anatomical studies of lymphatic drainage in the rat¹² have failed to reveal a lymphatic connection between the small bowel and cisterna chyli which does not pass through a mesenteric lymph node.

We conclude that in the mouse, mesenteric and peripheral small T lymphocytes form a common pool of cells which recirculate between the lymph nodes, spleen and Peyer's patches. The increased localisation of mesenteric small T lymphocytes to the liver may reflect the existence of slight differences in the composition of T-cell subsets between mesenteric and peripheral lymph nodes, as it has been found that ⁵¹Cr-labelled Ly 23 cells have an enhanced migration to the liver when compared with Ly 1 cells or with total unseparated T cells (de Sousa *et al.* in preparation).

We thank Mr J. Bognacki and Mrs H. Collin for technical assistance. This work was supported by the Special Projects Committee of the Memorial Sloan-Kettering Cancer Center and by the Medical Research Council.

ANTONIO A. DE FREITAS

Memorial Sloan-Kettering Cancer Center,
York Avenue,
New York, New York 10021

MARLENE L. ROSE*

DELPHINE M. V. PARROTT

Department of Bacteriology and Immunology,
Western Infirmary,
Glasgow G11, UK

Received 26 July; accepted 18 October 1977.

*Present address and to whom reprint requests should be sent: Chester Beatty Research Institute, Fulham Road, London SW3, UK.

1. Griscelli, C., Vassalli, P. & McCluskey, R. T. *J. Exp. Med.* **130**, 1427-1451 (1969).
2. Guy-Grand, D., Griscelli, C. & Vassalli, P. *Eur. J. Immunol.* **4**, 435-443 (1974).
3. Parrott, D. M. V. & Ferguson, A. *Immunology* **26**, 571-588 (1974).
4. McWilliams, M., Phillips-Quagliata, J. M. & Lamm, M. E. *J. Immunol.* **115**, 54-58 (1975).
5. Rose, M. L., Parrott, D. M. V. & Bruce, R. G. *Cell. Immunol.* **27**, 36-46 (1976).
6. Hopkins, J. & Hall, J. G. *Nature* **259**, 308-309 (1975).
7. Freitas, A. A. & de Sousa, M. *Eur. J. Immunol.* **5**, 831-838 (1975).
8. Julius, M. H., Simpson, E. & Herzenberg, L. A. *Eur. J. Immunol.* **3**, 645-649 (1973).
9. Tse, H. & Dutton, R. W. *J. Exp. Med.* **143**, 1199-1210 (1976).
10. Cahill, R. N. P., Poskitt, D. C., Frost, H. & Trnka, Z. *J. Exp. Med.* **145**, 420-428 (1977).
11. Sprent, J. *Cell. Immunol.* **21**, 278-302 (1976).
12. Tilney, N. L. J. *Anat.* **109**, 369-383 (1971).

T cell receptor idiotypes are controlled by genes in the heavy chain linkage group and the major histocompatibility complex

THE molecular identity of the receptor for antigen on T cells is so far only partially defined in that the variable region of the immunoglobulin (Ig) heavy chain is involved in its specificity-determining portion^{1,2}. All other parts of the receptor structure are at present only negatively defined: no variable region of an Ig light chain seems to be present, and the putative constant portions lack conventional immunoglobulin constant region determinants^{2,3}.

In another set of experiments soluble T cell-derived 'factors' have been described that possess both antigen-binding specificity and antigenic determinants encoded in the I-region of the *H-2* complex^{4,5}. These data in conjunction with the Ir-gene control of T cell function and the specificity differences between B and T cells suggest a model of a T cell receptor composed of one *Ig-I* coded and one *H-2* coded polypeptide⁶. If this model is correct, idotype

expression on T cells should be controlled by *VH* genes linked to allotype as well as by the *H-2* complex.

This question was investigated in mice using anti-idiotypic sera against idiotypic determinants of receptors on T cell blasts activated by mixed lymphocyte reaction (MLR). The anti-sera were raised as described⁷ by repeated injection of highly purified C57B1/6 (B6) HLR-activated AKR-T cell blasts into (AKRxB6) F1 hybrid mice. AKR responder cells were highly purified lymph node T cells (over 99.5% Ig-negative by indirect fluorescence). It is only in these circumstances that the activated T cell blasts are devoid of B cell immunoglobulin, an essential requirement for the generation of anti-idiotypic antibodies to T cell receptors only. The antiserum, which was directed against AKR receptors for B6, shortly F1a (AKRaB6), was absorbed with AKR-T cell blasts activated by SJL, to remove the previously described⁷ 'contaminating' antibodies against determinants on T cell blast membranes not associated with antigen receptors. The anti-idiotypic specificity of the absorbed F1a (AKRaB6) was shown by three methods. (1) In indirect immunofluorescence, the absorbed antiserum stained only B6-activated AKR- and (AKRxSJL) F1-T cell blasts; no staining was observed with SJL-activated AKR- and (AKRxB6) F1-T cell blasts. Con A-activated splenic AKR-T cell blasts did not stain above background. (2) In competition experiments we could show that soluble B6 alloantigens inhibited the uptake of F1a (AKRaB6) antibodies to trypsinised B6 MLR-activated AKR-T cell blasts after recovery from trypsin treatment, probably by blocking the access of the anti-idiotypic antibodies to the AKR receptors for B6 alloantigens. Only marginal blockade was observed with SJL and AKR control alloantigens. A detailed account of these experiments has been reported elsewhere⁷. (3) Idiotypic specificity was also observed upon addition of the antiserum to MLR cultures resulting in specific inhibition of T cell proliferation. We therefore concluded that the absorbed F1a (AKRaB6) reacted specifically with AKR T cell receptors for B6 alloantigens⁷.

The expression of the idiotypic determinants detected by F1a (AKRaB6) showed strain specificity, that is B6 MLR-activated AKR-T cell blasts expressed idotype whereas B6 MLR-activated SJL-T cell blasts did not (Table 1). The fact that AKR and SJL MLR responders differ in the *H-2* complex as well as by the *Ig-I* allotype (*H-2^k*, *Ig-I^d* and *H-2^s*, *Ig-I^b*, respectively) facilitated the study of the linkage of T cell receptor idotype expression to the *H-2* and/or *Ig-I* complexes.

Four groups of mice that differed with respect to *H-2* type and *Ig-I* allotype, were obtained by typing^{8,9} the offspring of a backcross of (AKRxSJL) F1 hybrid mice to SJL mice (Table 2): (1) *H-2^{s/s}*, *Ig-I^{b/b}*; (2) *H-2^{s/s}*, *Ig-I^{b/d}*; (3) *H-2^{s/k}*, *Ig-I^{b/b}* and (4) *H-2^{s/k}*, *Ig-I^{b/d}*. Highly purified lymph node T cells, pooled from four mice in each group, were separately activated in MLR by irradiated B6 lymph node stimulator cells. The resulting T cell blasts were purified and tested for the expression of idotypic determinants by indirect fluorescence with specifically absorbed F1a (AKRaB6). All fluorescent slides were read blind. As can be seen from Table 2, groups 1, 2 and 3 showed a low level of staining which we attribute to insufficient absorption of 'contaminating' antibodies. In marked contrast, group 4 was definitely and markedly positive. Only the mice of this group carried both the *H-2^k* haplotype as well as the *Ig-I^d* allotype of the idotype-positive parental strain AKR. No intermediate level of staining was observed in the groups that carried either the *Ig-I^d* allotype (2) or the *H-2^k* haplotype (3). This strongly suggests that the expression of T cell receptor idiotypes on MLR-activated T cell blasts in mice is controlled by two genes, one in the major histocompatibility complex (MHC) and the other in the *Ig-I* complex. It was surprising that the percentage of stained blasts in the doubly heterozygous group 4 is similar to that of homozygous parental AKR mice, and the question of allelic exclusion is presently under investigation.

The above data have to be discussed in conjunction with two earlier observations. Hämmerling *et al.*¹⁰ and Krawinkel *et al.*² reported that guinea pig anti-idiotypic antibodies to A5A idotype bearing anti-A-CHO antibodies of A/J mice react with helper T cells that possess A-CHO specificity, and that this reaction was

Table 1 Strain specificity of T cell receptor idiomorph expression on B6 MLR-activated AKR and SJL responder T cell blasts.

Responder	T cell blasts from MLR*	Stimulator	Positive responder T cell blasts (%)† with F1a (AKRxB6) anti-idiotypic serum‡
AKR (H-2 ^k , Ig-1 ^d)		B6	35.1%
SJL (H-2 ^s , Ig-1 ^b)		B6	5.0%

*Responder cells were nylon wool column passaged nonadherent lymph node cells (over 99.5% immunoglobulin negative by immunofluorescence; 38% (AKR) and 48% (SJL) recovery of cells from the nylon wool column). Stimulator cells were 3300 R irradiated lymph node cells. The conditions of the MLR and the nylon wool column separation have been described^{1,3}. AKR (responder, R), B6 (stimulator, S); stimulation index, S.J.: 616; over 99% blasts. SJL (R), B6 (S): S.J. 8.5; 91% blasts (over 99% T cells).

†Between 200 and 300 T cell blasts were counted. Blasts were recovered from the MLR by the Ficoll Urovison technique as described^{1,3}. Blast cells were defined as cells with a diameter at least twice that of a small lymphocyte, with a smaller nuclear to cytoplasmic ratio than small lymphocytes and with a nonsegmented nucleus (in distinction to nonlymphoid cells). Ig⁺ T⁺ blasts were detected by treating the cells simultaneously with rabbit anti-mouse T cell serum¹⁴ (dilution 1/400) and the mouse antiserum detected by tetramethyl-rhodamine isothiocyanate conjugated sheep anti-rabbit immunoglobulin G and fluorescein isothiocyanate conjugated sheep anti-mouse immunoglobulin G. All sera as well as all fluorescent conjugates were centrifuged at 16,000g for 10 min immediately before use. The exact procedure for fluorescent staining, preparation and labelling of anti-Ig antibodies, and fluorescence microscopy have been described^{7,13,15}.

‡The F1a (AKRxB6) anti-idiotypic serum was raised as described⁷. The antiserum was rendered specific by absorption with SJL MLR-activated AKR-T cell blasts, and used at a final dilution of 1/20. Control staining with normal (AKRxB6) F1 serum was below 1.5%.

restricted to T cells of those strains whose antibodies expressed the A5A idiomorph. Since A5A idiomorph expression is genetically linked to *Ig-1*⁹ and unlinked to *H-2*¹⁰, it was concluded from these studies that T cell receptor idiomorph expression is controlled by genes in the *Ig-1* complex and not by genes in the MHC. Because in these studies the anti-idiotypic antibodies were produced against an antibody molecule, whereas the anti-idiotypic antibodies used in the present experiments are made against T cell receptors directly, the two sets of data are not contradictory. Antisera produced against antibody molecules are not expected to react with T cell idiomorph determinants other than those shared with immunoglobulins and, consequently, cannot reveal any linkage to genes other than to those encoding immunoglobulin polypeptide chains.

Conversely, anti-idiotypic antisera produced against T cell receptors are expected to reveal idiomorph determinants unique to

unaltered set with allo-reactivity. Thus, strains of mice that differ in their *H-2* antigens would possess different anti-self sets and, consequently, different allo-reactive sets. Phenotypically this situation clearly results in the linkage of genes controlling allo-reactive receptors to the *H-2* complex. However, as the number of allo-antigens by far exceeds the number of self-antigens, it is reasonable to assume that the receptor sets with allo-reactivity partially overlap between different inbred strains. Thus, certain strain combinations with different *H-2* types should share allo-antigen receptors, and in these cases no *H-2* linkage would be observed. This may be an explanation for the results of Binz *et al.*¹¹ mentioned above.

A more direct way to account for MHC control of T cell idiomorphs is that genes in *H-2* directly encode peptides that are part of the receptor structure, as has been discussed in detail before⁶. This participation can take place either by a physical

Table 2 Linkage of T cell receptor idiomorph to the *H-2* and the immunoglobulin-1 locus

Responder†	T cell blasts from MLR*	Stimulator	Positive responder T cell blasts with F1a (AKRxB6) anti-idiotypic serum (%)
Group 1 (H-2 ^{s/k} , Ig-1 ^{b/b})		B6	6.2%
Group 2 (H-2 ^{s/s} , Ig-1 ^{b/d})		B6	6.6%
Group 3 (H-2 ^{s/k} , Ig-1 ^{b/b})		B6	6.3%
Group 4 (H-2 ^{s/k} , Ig-1 ^{b/d})		B6	40.6%

The experimental conditions for immunofluorescence are the same as in Table 1. All fluorescent slides were read blind. Control staining with normal (AKRxB6) F1 serum: 1.7% (group 1), 0.6% (group 2), 0.6% (group 3), and 4.6% (group 4).

*Responder cells were nylon wool column passaged nonadherent lymph node cells (over 99% immunoglobulin negative by immunofluorescence; 40% (group 1), 64% (group 2), 48% (group 3), and 56% (group 4) recovery of cells from the nylon wool column. Group 1 (R), B6 (S): S.J. 143; group 2 (R), B6 (S): S.J. 319; group 3 (R), B6 (S): S.J. 64; group 4 (R), B6 (S): S.J. 128.

†Each group consisted of cells from a pool of 4 mice *H-2* typed and allotyped as described^{8,9}.

T cells, should they exist. In this context, Binz and Wigzell have published a series of experiments showing that anti-idiotypic antibodies against rat T cell alloantigen-receptors do not reveal any idiomorph determinants unique to T cells and, consequently, do not reveal any linkage of T cell idiomorph-controlling genes to genes other than those controlling immunoglobulin heavy chains^{1,11}. These data suggested that the T cell receptor is constructed from *VH* regions only.

In contrast, the data presented in this paper clearly show that T cell receptor idiomorphs are controlled by two unlinked genes. One of these genes is located in the heavy chain linkage group (*Ig-1* complex) and all the available evidence^{1,2,3,10,11} suggests that this directly encodes a heavy chain *V* region peptide that is part of the T cell receptor. The control exerted by the second, *H-2* linked, gene(s) can be envisaged in at least two different ways. MHC-coded antigens could play an essential role in the generation of diversity in the sense suggested by Jerne¹² who proposed that the immunological repertoire is composed of two sets of specific receptors, one derived by mutation and selection from a set of receptors with anti-self specificity, and the other a relatively

association of the *H-2* product with *VH*, or by the existence of a separate class of *H-2* coded receptors in addition to the *VH*-containing receptors. The latter possibility seems unlikely because in the present experiments no intermediate phenotypes have been observed in the mice carrying either the appropriate *H-2* allele or the appropriate allotype.

For the moment, we cannot distinguish between an indirect regulatory influence of *H-2* antigens by selection of a given receptor repertoire, and a direct coding of genes in the *H-2* complex for parts of the T cell receptor. We may, however, generalise in concluding that the T cell repertoire differs from that of B cells, either by virtue of physical differences in the combining sites or by virtue of different routes of selection in ontogeny.

We thank D. Mohr and I. Falk for technical assistance.

PETER H. KRAMMER
KLAUS EICHMANN

*Institut für Immunologie und Genetik,
Deutsches Krebsforschungszentrum,
Im Neuenheimer Feld 280,
D-6900 Heidelberg, Germany*

Received September 23; accepted October 18 1977.

1. Binz, H. & Wigzell, H. *Cold Spring Harbor Symp. quant. Biol.* **61**, 275-284 (1976).
2. Krawinkel, U. et al. *Cold Spring Harbor Symp. quant. Biol.* **61**, 285-294 (1976).
3. Eichmann, K. in *The Immune System: Genes and the Cells in which they Function* (eds Sercarz, E., Herzenberg, L. & Fox, F.) (Academic Press, New York, in the press).
4. Taussig, M. J., Munro, A. J., Campbell, R., David, C. G. & Stained, N. A. *J. exp. med.* **142**, 694-712 (1975).
5. Tada, T., Taniguchi, M. & David, C. G. *J. exp. med.* **144**, 713-731 (1976).
6. Rajewsky, K. & Eichmann, K. in *Contemporary Topics in Immunobiology 7: T cells* (ed. Stutman, O.) (Plenum Press, New York, 1977).
7. Krammer, P. H. *J. exp. med.* (in the press).
8. Weissstein, P. J., Krammer, P., Nowinsky, R. C., David, C. S., Frelinger, J. A. & Shreffler, D. C. *Immunogenetics* **3**, 507-516 (1976).
9. Eichmann, K. & Berek, C. *Eur. J. Immun.* **3**, 599-601 (1973).
10. Hammerling, G., Black, S. J., Berek, C., Eichmann, K. & Rajewsky, K. *J. exp. Med.* **143**, 861-879 (1976).
11. Binz, H., Wigzell, H. & Bazin, H. *Nature* **264**, 639-642 (1976).
12. Jerne, N. K. *Eur. J. Immun.* **1**, 1-9 (1972).
13. Nagy, Z., Elliott, B. E., Nabholz, M., Krammer, P. H. & Pernis, B. J. *J. exp. Med.* **143**, 648-659 (1976).
14. Sauter, D., Anckers, C. & Bron, C. *J. Immun.* **113**, 617-624 (1974).
15. Krammer, P. H., Citronbaum, R., Read, S. E., Forni, L. & Lang, R. *Cell. Immun.* **21**, 97-111 (1976).

Depression of macrophages in mice drinking hyperchlorinated water

WE have encountered a problem with the collection and *in vitro* activation of peritoneal exudate macrophages (PEM) from mice, which we can now associate with high levels of chlorine in the drinking water. Historically the chlorination of drinking water in colonies of experimental rodents was carried out in order to avoid the so-called early death syndrome in lethally irradiated animals, which is due to pathogenic enteric bacteria such as *Pseudomonas*. The recommended level of chlorine necessary to achieve this goal is 12-16 parts per million (p.p.m.)¹. Because of an unusually high incidence of *Pseudomonas* within a few breeding units of our animal facility, the chlorination level of the drinking water was increased to 25-30 p.p.m. We found that this hyperchlorination had an adverse effect on an important host defense mechanism, that is the macrophage system, whose primary function is to eliminate microbial pathogens^{2,3} and neoplasms^{3,4}.

Macrophages that normally reside in the peritoneal cavity and those that are induced to accumulate there by the intraperitoneal (i.p.) injection of inflammatory agents are not cytotoxic to tumour cells *in vitro*⁵⁻⁸. Such PEM can be rendered tumoricidal (TM) by bacterial products, endotoxins, pyran copolymer and double-stranded RNA, chronic infection of animals with obligate intracellular bacteria, or certain protozoa^{7,8}. Macrophages can also be rendered tumoricidal by incubation with soluble mediators (lymphokines) released by antigen- or mitogen-stimulated lymphocytes, referred to as 'macrophage activating factor' (MAF)⁷⁻¹¹.

Tumoricidal activity can also result as a consequence of a host immune response against syngeneic tumours *in vivo*^{5,6,12}. Recently, we found that PEM collected from tumour-immunised mice was not tumoricidal *in vitro*, and PEM from normal mice could not be rendered tumoricidal *in vitro* by incubation with MAF. Since the collection and culture of PEM, and cytotoxicity assays using PEM are influenced by a variety of factors^{4,6,7}, we first attempted to overcome our difficulties by varying the media, serum lot, type of inflammatory agent, and so on; all to no avail. We then examined the daily records of our animal facility and noticed that the levels of chlorine in the drinking water had been increased from 12-15 p.p.m. to 25-30 p.p.m. This increase seemed to coincide with the emergence of our difficulties. We therefore set out to investigate the possibility that hyperchlorination of drinking water could adversely affect murine PEM.

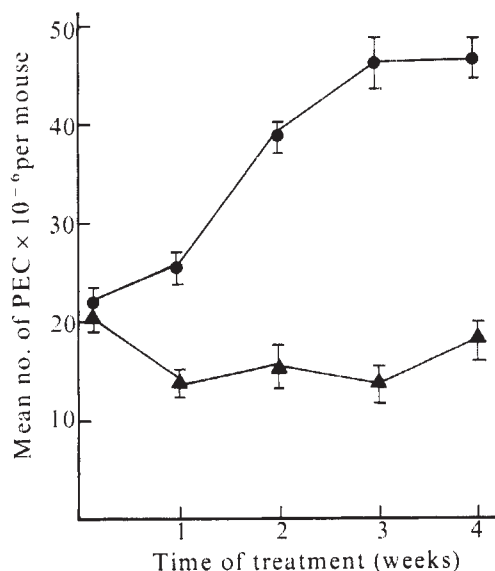
C57BL/6N female mice weaned at 3-4 weeks of age were given sterile (and free of *Pseudomonas*) tap water (0.5-1.0 p.p.m. chlorine) for 2 weeks. When the mice were 5-6 weeks old they were divided into two groups. The first group

received hyperchlorinated drinking water (25-30 p.p.m.) and the second group continued on tap water and served as controls. Chlorine and chloramine were assayed by the *O*-toluidine method¹³ by which the total chlorine is measured (OCI^- , HOCl , NH_2Cl , NHCl_2 and NCl_3). Chlorinated drinking water was prepared (as is the routine procedure in our animal colony) twice weekly by adding a solution of sodium hypochlorite to tap water. At weekly intervals groups of five mice each were injected i.p. with 2 ml of thioglycollate broth. Individual mouse PEC differential and total count was carried out 5 d later. The results of a representative experiment are shown in Fig. 1. At the start of this experiment and just before treatment, the mean number of PEC per mouse was $21 \pm 4 \times 10^6$. One week later the number of PEC obtained from mice receiving hyperchlorinated water had decreased to $13 \pm 2 \times 10^6$ per mouse, whereas control mice yielded $25 \pm 3 \times 10^6$ PEC ($P < 0.001$). On consecutive weeks, the PEC yield from control mice increased, but the PEC yield from mice receiving hyperchlorinated water remained low.

At each week, PEM from mice receiving hyperchlorinated water or tap water were assayed for *in vitro* tumoricidal properties. The adherent PEM cultures were incubated *in vitro* with supernatants obtained from cultures of rat lymphocytes stimulated with Sepharose-bound concanavalin A (Pharmacia) which were previously shown to contain MAF activity (Con A-MAF)¹⁰. Macrophage-mediated cytotoxicity *in vitro* was measured by release of radioactivity from pre-labelled target cells^{8,10,12} as described in the legend to Table 1. Tumour cell killing *in vitro* was measured by release of radioactivity (^{125}I -UdR) from pre-labelled syngeneic tumour cells, the B16 melanoma and ultraviolet-induced fibrosarcoma. Results of a representative experiment are shown in Table 1.

PEM from mice receiving tap water or hyperchlorinated water were not significantly cytotoxic to either syngeneic tumour target, which is in agreement with our previous findings^{5,10,12}, and those of others^{3,4,6-9,16,20}. The PEM from mice receiving tap water were cytotoxic to both syngeneic tumour targets following their treatment with Con A-MAF. The levels of specific cytotoxicity differed slightly from week to week and ranged from 43 to 68% for the B16 melanoma and 62-76% for the UV-112 fibrosarcoma ($P < 0.001$). In contrast, PEM collected from hyperchlorinated mice exhibited significantly lower levels of cytotoxicity ($P < 0.01$) during the first two weeks of treatment

Fig. 1 Mean no. of peritoneal exudate cells collected from mice receiving tap (0.5 p.p.m. chlorine ●) or hyperchlorinated (25-30 p.p.m. ▲) drinking water. At any time point, the differences between the groups were highly significant ($P < 0.001$).



(28–31% for the B16 melanoma and 24–48% for the UV-112 fibrosarcoma). By the third week of treatment, these PEM (even though treated with Con A-MAF) were not tumoricidal.

These studies show that the addition of high levels of chlorine in the drinking water of mice produces profound alterations in numbers of PEM and tumoricidal function. The hyperchlorination of drinking water in facilities housing laboratory animals is a common occurrence. The effects of 25–30 p.p.m. of chlorine become apparent within a matter of weeks. It is possible that the lower level (10–15 p.p.m.) of chlorine also exerts such effects, but over a much longer period of time. Supporting this possibility is the report that urban water (chlorinated at 2–4 p.p.m.) filtered by reverse osmosis and used in long-term haemodialysis of human patients was shown to cause acute haemolytic anaemia. Chlorine compounds brought about denaturation of haemoglobin by direct oxidation and also by the inhibition of the direct oxidative pathway, hexose monophosphate shunt (HMPS), of red blood cell (RBC) metabolism. This damage to RBC in haemodialysed patients was found to be cumulative over several periods of dialysis¹⁴.

The mechanism by which hyperchlorinated water affects murine macrophages is unknown. Several possibilities may be considered. The vacuolar system of macrophages is probably involved in the mechanism of cytotoxicity^{8,15}. Inhibition of macrophage lysosomal enzymes by Trypan

blue and stabilisation of lysosomal membranes by hydrocortisone have been shown to suppress macrophage cytotoxic activity¹⁶. Moreover, the translocation of lysosomes from tumoricidal macrophages into susceptible target cells has been demonstrated by phase contrast¹⁶ and electron microscopic studies¹⁷. Lymphokine-treated macrophages, which have enhanced bactericidal capacity¹⁸, were found to have a 4- to 8-fold increase in glucose oxidation compared to untreated control macrophages. Nearly all the observed glucose oxidation was attributed to metabolism through the direct oxidative pathway (HMPS)¹⁹. An inhibition of HMPS metabolism in macrophages by chlorine compounds¹⁴ could perhaps reduce their tumoricidal activity. Chlorine compounds may also affect macrophages indirectly. Pathological conditions that produce large numbers of damaged RBC, haemoglobin or haemoglobin degradation products within the macrophage vacuolar system (lysosomes) have now been shown to suppress macrophage tumoricidal activity¹⁵. As stated above, chlorine compounds have been shown to cause severe methaemoglobinemia and haemolysis¹⁴.

Regardless of the mechanism by which chlorinated water suppresses macrophages, the consequences of this suppression could be quite far reaching. The macrophage is thought to play a major part in host defence against neoplasia^{20–22}. Further, substances known to depress macrophage function such as silica, carageenan and Trypan blue are reported to decrease host resistance against transplantable tumours^{20,21}. These findings and the experiments reported here raise the possibility that host resistance against neoplasms may also be compromised by hyperchlorinated drinking water.

This work was supported by the US NCI (contract N01-C0-25423 with Litton Bionetics). I thank Dr M. L. Kripke for discussions and Mr William Fogler and Ms Zoa Barnes for technical assistance.

ISAIAH J. FIDLER

Basic Research Program,
NCI-Frederick Cancer Research Center,
P.O. Box B,
Frederick, Maryland 21701

Received 8 August; accepted 24 October 1977.

1. Simmons, M. L. & Brick, O. J. in *The Laboratory Mouse Selection and Management* (ed. Hollaender, A.) 67–69 (Prentice Hall, Englewood Cliffs, 1969).
2. Mackaness, G. B. in *Infectious Agents and Host Reactions* (ed. Mudd, S.) 62–78 (Saunders, Philadelphia, 1970).
3. Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Nature new Biol.* 235, 48–50 (1972).
4. Hibbs, J. B., Jr, Taintor, R. R., Chapman, H. A. & Weinberg, J. B. *Science* 197, 279–282 (1977).
5. Fidler, I. J. *J. natn. Cancer Inst.* 55, 1159–1163 (1975).
6. Russell, S. W. & McIntosh, A. T. *Nature* 268, 69–71 (1977).
7. Evans, R. & Alexander, P. in *Immunobiology of the Macrophage* (ed. Nelson, D. S.) 536–573 (Academic, London, 1976).
8. Hibbs, J. B., Jr *J. natn. Cancer Inst.* 53, 1487–1492 (1974).
9. Piessens, W. F., Churchill, W. J., Jr & David, J. R. *J. Immun.* 114, 293–299 (1975).
10. Fidler, I. J., Darnell, J. H. & Budmen, M. B. *Cancer Res.* 36, 3608–3615 (1976).
11. Fidler, I. J., Darnell, J. H. & Budmen, M. B. *J. Immun.* 117, 666–673 (1976).
12. Kripke, M. L., Budmen, M. B. & Fidler, I. J. *Cell. Immun.* 30, 341–352 (1977).
13. Sawyer, C. N. & McCarthy, P. L. *Chemistry for Sanitary Engineers* (McGraw-Hill, New York, 1964).
14. Eaton, J. W., Kolpin, C. F., Swofford, J. S., Kjellstrand, C. M. & Jacobs, H. S. *Science* 181, 463–464 (1973).
15. Weinberg, J. B. & Hibbs, J. B., Jr *Nature* 269, 245–247 (1977).
16. Hibbs, J. B., Jr *Science* 184, 468–471 (1974).
17. Bucana, C. et al. *Cancer Res.* 36, 4444–4458 (1976).
18. Nathan, C. F., Karnovsky, M. L. & David, J. R. *J. exp. Med.* 133, 1356–1364 (1971).
19. David, J. R. in *Progress in Immun.* (ed. Amos, B.) 399–412 (Academic, New York and London, 1971).
20. Hibbs, J. B., Jr *Transplantation* 19, 77–81 (1975).
21. Keller, R. J. *J. natn. Cancer Inst.* 57, 1355–1361 (1976).

In vitro culture reduces immunogenicity of pancreatic endocrine islets

HUMAN pancreatic transplantation results, using the whole organ, have been disappointing and interest has been focused recently on the promising results obtained by trans-

Table 1 *In vitro* cytotoxicity mediated by MAF-treated and untreated macrophages from C57BL/6 mice receiving hyperchlorinated or tap drinking water

Week of treatment	Drinking water*	% Macrophage-mediated Cytotoxicity † against	
		B16 Melanoma	UV-112 Fibrosarcoma
1	Tap	55‡	76‡
	Hyperchlorinated	28§	48‡
2	Tap	68‡	70‡
	Hyperchlorinated	31§	24§
3	Tap	43‡	62‡
	Hyperchlorinated	0	0
4	Tap	48‡	72‡
	Hyperchlorinated	0	0

*Tap water: 0.5–1.0 p.p.m. of chlorine. Hyperchlorinated: 25–30 p.p.m. of chlorine.

†PEC (suspended in supplemented media) were plated into 60 × 15 mm plastic dishes at a concentration of 2×10^6 per dish. Thirty min after incubation at 37 °C, non-adherent PEC were removed by washing with media, and fresh media was added. The cultures were then incubated for an additional 24 h. At this time, all the adherent cells from mice receiving tap or hyperchlorinated water were phagocytic and morphologically resembled macrophages. The plating efficiencies and differential counts of PEC collected from the two treatment groups were similar throughout the experiments. The B16 melanoma and fibrosarcoma UV-112 tumour target cells from the C57BL/6 mouse strain were labelled *in vitro* for 24 h with 0.3 µCi/ml of ¹²⁵I-UdR (200 mCi/µmol⁻¹; New England Nuclear). The tumour cultures were washed with media to remove unincorporated radioactive label and cells were collected. 1×10^4 labelled target cells were plated alone or were added to the Con A-MAF treated or untreated macrophage cultures, making an initial ratio of 100:1 adherent PEM to target cells. After 24 h, the triplicate cultures were washed and re-fed with media to remove target cells that did not plate. On day 5, the cultures were washed twice to remove non-adherent cells, and the remaining viable cells were lysed with 1 ml 0.5 M NaOH. The lysate and two rinses were combined and counted in a gamma counter. The plating efficiencies of tumour cells plated alone or on macrophage cultures have been previously shown to be similar^{8,10}. The % cytotoxicity in the macrophage assays was computed with the following formula: [(c.p.m. of target cells with normal macrophages – c.p.m. of target cells with TM)/c.p.m. of target cells with normal macrophages] × 100. The statistical significance of differences between groups was tested with Student's two-tailed *t*-test.

‡*P* < 0.001.

§*P* < 0.02.

planting isolated endocrine islets. Islet cell is transplantation can restore the diabetic animal successfully to a state of normoglycaemia^{1,2}. Unfortunately, islet allografts have been associated with only brief periods (4–8 d) of normoglycaemia, and attempts to prolong function with immunosuppressive therapy of the host have generally been unsuccessful³. The present results, however, suggest that the survival time of pancreatic islets, transplanted directly into the liver of a histoincompatible recipient, is prolonged by *in vitro* culture of the tissue before transplantation. Complete or partial control lasting up to more than 160 d was achieved in 70% of recipients. Nevertheless, allogeneic cultured transplants are less effective in equivalent quantities than isogeneic grafts, in reversing an experimental diabetic state in rats.

Evidence has accumulated that the immunogenicity of certain types of allografts is to some extent amenable to experimental manipulation⁴. Organ culture before transplantation may deplete the tissue of viable haematogenous elements and lymphoid cells^{5,6} and/or diminish the concentration or availability of stimulatory antigens on the cell surface membranes⁷. In fact, as early as 1934, Stone *et al.*⁸ attempted to adapt endocrine tissue in organ culture before transplantation. Other investigations with parathyroid, adrenal and pituitary organ-cultured transplants have shown that a growth period *in vitro* delays an adverse response of the host to the grafts⁹. Up to now, in spite of conflicting data about the enhancing effect of *in vitro* culture on the survival of skin grafts¹⁰, well controlled experiments have established that as a result of maintenance *in vitro* for 3 to 13 d, a significant proportion of allografts of ovarian and thyroid tissues are able to override major histocompatibility barriers^{11,12}. Apart from one study mentioned in a preliminary abstract¹³ no previous report deals with enhancement of survival of endocrine pancreas allografts after organ culture.

We have studied whether *in vitro* culture before transplantation enhances the survival of pancreatic endocrine allografts and have confirmed the functional value of cultured endocrine islets¹⁴. The endocrine pancreatic grafts

were transplanted directly into the anterior hepatic lobe of the recipient, since several investigators have suggested that the liver may exert a protective role on allograft tolerance (for reviews see refs 15–17). Diabetes was produced in young adult inbred male Lewis (LW) rats of 200 g body weight (Streptozotocin -IV- 65 mg kg⁻¹). The rats received food and water *ad libitum* and each animal was isolated in a separate metabolic cage. Determination of body weight, daily measurements of urine volume and glucose content, and twice weekly measurements of serum glucose and insulin concentration were made. No animal was considered hyperglycaemic unless non-fasting blood glucose levels were over 4 g% on the six determinations over a period of at least 15–18 d. Isolated endocrine islets from Wistar (WAG) inbred rats prepared by the classical collagenase digestion were collected under the dissecting microscope. One thousand islets, originating from three donors represented the amount of endocrine tissue grafted in each recipient. The islets were either transplanted immediately after their isolation or after culture for 4 or 5 d in Falcon dishes containing CMRL-1066 medium supplemented with 10% heat-inactivated calf serum¹⁴. Pancreatic transplantation of cultured or non-cultured endocrine islets was carried out by injecting the suspension of 1,000 islets in 0.5 ml of culture medium directly into the anterior hepatic lobe of the recipient. Neither exogenous insulin nor additional immunosuppressive therapy was administered to the recipients. The two inbred rat strains used were histoincompatible at the major locus (LW:Rt H-1ⁱ and WAG:H-1^w) and this has been confirmed by cross skin grafts between donor and recipient strains.

As previously shown with mouse islets¹⁴, the culture of rat islets at 5 mM glucose concentration for 4 d led to a 50–60% decrease in the islet insulin content. The functional response of the cultured islets has been investigated (Fig. 1). Short term experiments (90 min) showed that 4-d cultured islets lost their sensitivity to glucose stimulation, compared with fresh islets. Insulin release by cultured islets, however, was stimulated by glucose alone or with glucagon, during prolonged incubation (24 h). Moreover, in these latter conditions the total insulin content (islets + medium) was always higher than that of the 4-d cultured islets (16.84 ± 3.2 mU per 20 islets), suggesting insulin synthesis by the cultured islets.

In animals treated with Streptozotocin (36 rats) the mean survival time (± s.e.m.) was about 25.5 ± 2.6 d following induction of the experimental diabetes. In animals receiving allogeneic non-cultured islet transplants (13 cases), the mean survival time was prolonged to 56.0 ± 7.3 d. Culture before transplantation increased the survival of the grafted animals significantly ($P < 0.001$) (13 cases) to more than 161 d. Three of these animals are still surviving more than 6 months after the graft.

The biochemical and metabolic progress of the grafted animals has been compared with that we have observed previously² after isogeneic islet cell transplantation (Fig. 2). After allogeneic non-cultured islet transplantation, a temporary response was observed in 9 of 13 grafted animals. In the four remaining cases, the transplantation procedure did not significantly improve the diabetic state. This rate of success is similar to that observed previously in isogeneic islet transplantation². The nine positive grafts, however, were followed by reversal to the diabetic state after a mean time of about 8.22 ± 0.81 d. On the other hand, among the 13 recipients of cultured allografts, 11 responded well to the graft, and only two of these reversed to the diabetic state between the 10th and 20th post-transplant day, while in the other nine a functioning graft survived for at least 90 d without immunosuppressive therapy of the host. In these animals the fasting level of blood sugar was significantly improved as compared with pretransplant levels,

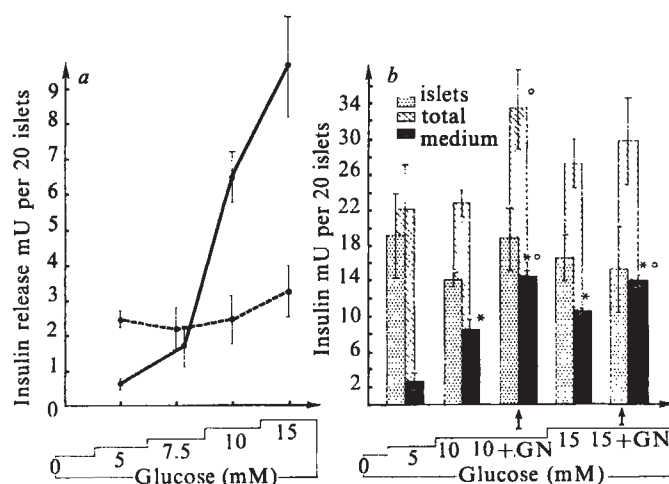


Fig. 1 Glucose-stimulated insulin release of cultured islets. *a*, Short term incubation (90 min) of 4-d cultured islets (---) and fresh islets (—). Note the insensitivity of the cultured islets to increasing glucose concentrations. *b*, Prolonged incubation (24 h) of 4-d cultured islets in the presence of increasing glucose concentrations and addition of 10 µg ml⁻¹ glucagon. Small non-significant variations of the insulin content of the islets are observed, whereas glucose (10 mM and 15 mM) significantly increased their insulin output. Further addition of glucagon potentiates the glucose effect. The results are expressed as mean values (mU per 20 islets ± s.e.m.) from 5 (*a*) to 10 (*b*) different experiments. Statistical significance was analysed by unpaired Student's *t* test; *, *t* significant against 5 mM glucose ($P < 0.001$); ○, *t* significant against glucose alone ($P < 0.05$). GN, Glucagon.

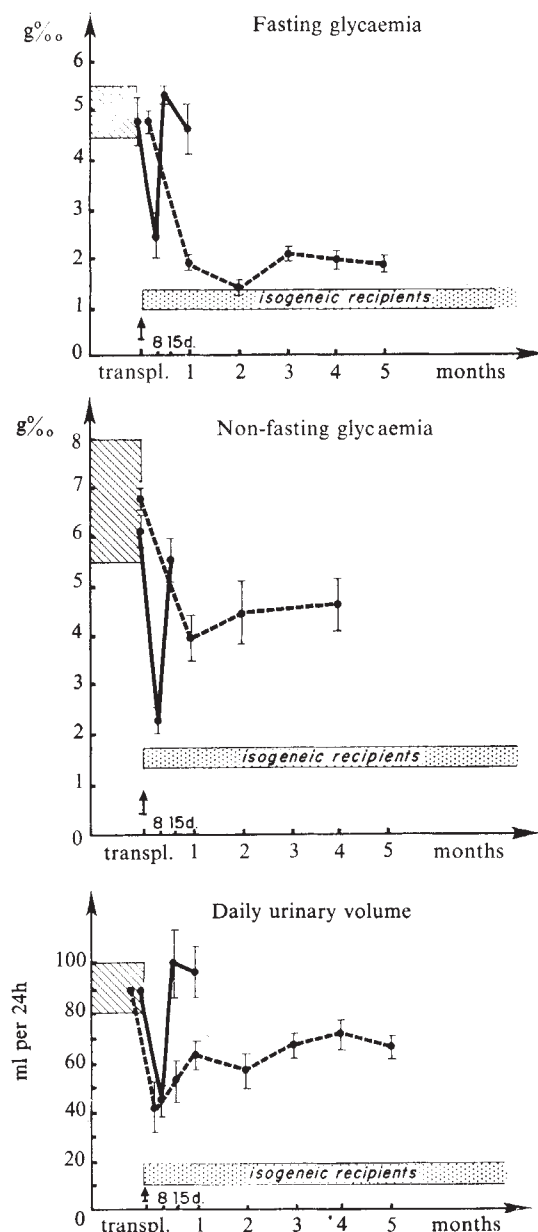


Fig. 2 Effect of transplantation of 1,000 islets on glycaemia and urinary volume of diabetic animals (shaded boxes) after: isogeneic islet transplantation (11 cases, dotted bar); non-cultured allogeneic islet transplantation (9 cases, —); 4-d cultured allogeneic islet transplantation (9 cases, ----).

but remained significantly different from non-diabetic animals and/or isogeneic recipients. Moreover, the rise in blood sugar after feeding as well as urine volume were not controlled within normal limits. Insulinaemia of these animals ranged between 13 and 30 $\mu\text{U ml}^{-1}$ at the 5th post-operative month and was not different from that observed at the 8th post-transplant day, but was significantly different from pretransplant levels ($P < 0.05$).

From these results we can say that *in vitro* culture before transplantation induces a fall in the insulin content of the islets. Allogeneic cultured transplants of endocrine tissue are functionally less effective in equivalent quantities than isogeneic grafts and are unable to control carbohydrate metabolism fully. Culture of the pancreatic islets, before transplantation, markedly enhances the host tolerance to the graft in the absence of immunosuppressive therapy and in spite of the existence of strong histoincompatible antigens between donor and recipient. Based on previous hypotheses and experimental results^{5,6} the loss of metabolically active lymphoid cells during pancreatic islet

culture is now under investigation. The protective role of the liver on graft survival does not seem to be confirmed and seems to be limited to weak antigen barriers.

This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (75-5.0207). Insulin determinations of cultured islets were performed by A. J. Moody (Novo Research Institute, Copenhagen) to whom we are grateful. Dr Pataud of the Upjohn Company kindly supplied Streptozotocin. We thank Eliane Munch and Francine Gosse for technical assistance.

MICHELE KEDINGER
KATY HAFFEN
JACQUES GRENIER
ROSY ELOY

Unit 61 INSERM,
Surgical Research and Biophysiopathology of
the Intestine,
Avenue Molière,
67200 Strasbourg/Hautepierre, France

Received 1 August; accepted 18 October 1977.

1. Ballinger, W. F. & Lacy, P. E. *Surgery* 72, 175-186 (1972).
2. Eloy, R. *et al. Horm. metab. Res.* 9, 40-46 (1977).
3. Finch, D. R. A. & Morris, P. J. *Transplantation* 22, 503-512 (1976).
4. Billingham, R. E. J. *invest. Derm.* 67, 149-159 (1976).
5. Lafferty, K. J., Cooley, M. A., Woolnough, J. & Walker, K. Z. *Science* 188, 259-261 (1975).
6. Bach, F. H., Bach, M. L. & Sondel, P. M. *Nature* 259, 273-281 (1976).
7. Opelz, G. & Terasaki, P. I. *Science* 184, 464-466 (1974).
8. Stone, H. B., Owings, J. C. & Gey, G. O. *Ann. Surg.* 199, 613-626 (1934).
9. Gaillard, P. J. *Ciba Fdn Symp.* 100-106 (Churchill, London, 1964).
10. Ninnemann, J. L. & Good, R. A. *Transplantation* 18, 1-5 (1974).
11. Jacobs, B. B. *Transplantation* 18, 454-457 (1974).
12. Lafferty, K. J., Cooley, M. A., Woolnough, J. & Warler, K. Z. *Science* 188, 259-261 (1975).
13. Boyles, R. R. & Seltzer, H. S. *Diabetes (Abstr.)* 24, 420 (1975).
14. Kedinger, M., Moody, A. J., Launay, J. F. & Haffen, K. *Experientia* 33, 972-974 (1977).
15. Eloy, R., Vuitton, D., Vaultier, J. P., Pousse, A. & Grenier, J. F. *Cell. Immun.* 21, 236-242 (1975).
16. Vuitton, D., Eloy, R., Coumaros, G. & Grenier, J. F. *Cell. Immun.* 28, 51-58 (1977).
17. Vuitton, D., Eloy, R., Clendinnen, G. & Grenier, J. F. *Cell. Immun.* (in the press).

Selective inhibition of response of *Ips pini* to its pheromone by the (S)-(—)-enantiomer of ipsenol

THE response of the bark beetle *Ips pini* (Say) (Coleoptera: Scolytidae) to the attractant produced by conspecific males boring in ponderosa pine is inhibited by the presence of boring males of *I. paraconfusus* Lanier¹. Ipsenol, 2-methyl-6-methylene-7-octen-4-ol, one of three synergistic components required to elicit attraction in *I. paraconfusus*², also reduces catches of *I. pini* at traps baited with boring male *I. pini*^{1,3}. Ipsenol has not been found in *I. pini*. Because (S)-(—)-ipenol is the only enantiomer of ipsenol present in *I. paraconfusus*⁴, we compared the inhibitory activity of the enantiomers of ipsenol with that of the racemic compound in the laboratory and field and we found that *I. pini* is affected specifically by the (S)-(—)-enantiomer.

We measured the walking response of *I. pini* in an open-arena olfactometer⁵ to aliquots of a cold-condensate of volatiles produced by 97 male *I. pini* while boring in ponderosa pine for 42 h (ref. 6). The attractant condensate was delivered in pentane from a power-driven syringe at about 5.5×10^{-2} beetle-minutes per min (one beetle-minute of condensate is equivalent to the attractant produced by one male boring for 1 min). The two enantiomers were synthesised from (S)-(+)-leucine and its antipode⁷, and both they and racemic ipsenol were delivered in pentane, by syringe, at $5 \times 10^{-6} \mu\text{l min}^{-1}$. Response was measured by the number of beetles reaching the source out of groups of ten released on the surface of the arena. All beetles used for assay were collected as they emerged in the laboratory from infested ponderosa pine and were stored in the same conditions before assays.

In the field, the response of *I. pini* was measured by the number trapped on cylindrical wire screen traps coated with a sticky material^{1,3}. The attractive standard was produced by introducing 21 (test 1) and 25 (test 2) male beetles into pre-drilled holes in small ponderosa pine logs (15 × 30 cm). Each log was wrapped in fine metal screen to prevent attacks by responding beetles, and was placed inside the sticky trap on a pipe standard 1 m above ground. The compounds were evaporated from 5-μl open-ended capillary tubes; one capillary suspended inside an inverted 35-mm film canister with a perforated lid. Two canisters were hung on opposite sides of a trap. The rates of evaporation of

Table 1 Inhibition of the response of female *I. pini* to condensates of volatiles produced by boring male *I. pini*, by enantiomers of ipsenol

Test material	Response		No. of assays
	Mean of 10	(s.e.)	
Condensate	5.07	(2.94) (a)	6
Condensate+(R)-(+)- ip-senol	2.62	(1.98) (b)	13
Condensate+(S)-(–)- ip-senol	0.83	(1.03) (c)	12
Condensate+racemic ip-senol	0.17	(0.41) (c)	15
No stimulus	0.17	(0.41) (c)	6

Tests were conducted between 1 and 13 September, 1976 in Davis, California. Responses followed by different letters are significantly different at $P < 0.05$ (Mann-Whitney test).

both enantiomers and racemic ipsenol, measured volumetrically in the field, were approximately 2 mg for 24 h for each treatment. Treatments were arranged in a line, 20 m apart. Beetles were picked from the traps which were rotated every 2 h, so that all treatments occupied all positions at least once.

The responses of female *I. pini* in the laboratory to cold condensate alone and to condensate delivered with (R)-(+)-ip-senol were both significantly higher than those to condensate delivered with either racemic ipsenol or (S)-(–)-ip-senol and to the control (no condensate or ipsenol) (Table 1). The slight, but significant, activity of (R)-(+)-ip-senol in inhibiting female response to cold condensate could easily have been due to contamination, which is difficult to avoid in that type of assay. Too few males were available to provide a significant result, but their response paralleled that of the females.

In field tests, (R)-(+)-ip-senol had no measurable effect on catches of *I. pini* at traps containing males boring in logs (Table 2). In contrast, on traps where racemic or (S)-(–)-ip-senol was evaporated beside a log containing males, there

Table 2 Effect of enantiomers and racemic ipsenol on catches of *I. pini* at traps containing male *I. pini* boring in ponderosa pine

Treatment	Test 1			Test 2		
	<i>I. pini</i> trapped			<i>I. pini</i> trapped		
	Total	Median	♂:♀	Total	Median	♂:♀
<i>I. pini</i>	47	3(a)	1:1.8	32	2(a)	1:0.8
<i>I. pini</i> + (R)-(+)-ip-senol	27	3(a)	1:1.25	43	3(a)	1:1.26
<i>I. pini</i> + (S)-(–)-ip-senol	1	0(b)	—	2	0(b)	0:2.0
<i>I. pini</i> + racemic ipsenol	8	0(b)	—	3	0(b)	3:0.0
Ponderosa pine log alone	0	0	—	3	0(b)	—
Racemic ipsenol alone	0	0	—	0	0	—

Test 1 was conducted between 6 and 9 July and test 2 between 17 and 21 July, 1977 at McCloud Flats, California. Eleven 2-h replications of each treatment were done in each test. There were 21 males per bolt in test 1 and 25 in test 2. Trap catches followed by different letters are significantly different (Wilcoxon signed rank test) at $P < 0.01$ (test 1) and $P < 0.05$ (test 2).

were so few catches that there was no significant difference from control traps containing either a log with no beetles or racemic ipsenol alone. Thus, the walking responses of *I. pini* measured in the laboratory paralleled the response in the field, although with less precision. From both sets of results we conclude that the inhibition of aggregation in *I. pini* by boring males of *I. paraconfusus* is probably due to (S)-(–)-ip-senol released by those males.

Although enantiomeric specificity of the components of attractant pheromones has been demonstrated in several species of Scolytidae, *Dendroctonus*⁸, *Gnathotrichus*⁹, and *Ips*^{10–12}, we believe that ours is the first demonstration of enantiomeric specificity of an interspecific pheromonal inhibitor in this family. The restricted production of and response to enantiomers is one mechanism which enhances the range of unique combinations of available olfactory stimuli.

M. C. BIRCH
D. M. LIGHT

Department of Entomology,
University of California,
Davis, California 95616

K. MORI

Department of Agricultural Chemistry,
University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

Received 26 July; accepted 28 October 1977.

1. Birch, M. C. & Wood, D. L. *J. chem. Ecol.* **1**, 101–113 (1975).
2. Silverstein, R. M., Rodin, J. O. & Wood, D. L. *Science* **154**, 509–510 (1966).
3. Birch, M. C. & Light, D. M. *J. chem. Ecol.* **3**, 257–267 (1977).
4. Mori, K. *Tetrahedron Lett.* **26**, 2187–2190 (1975).
5. Wood, D. L., Browne, L. E., Silverstein, R. M. & Rodin, J. O. *J. Insect Physiol.* **12**, 523–536 (1966).
6. Browne, L. E., Birch, M. C. & Wood, D. L. *J. Insect Physiol.* **20**, 183–193 (1974).
7. Mori, K. *Tetrahedron*, **32**, 1101–1106 (1976).
8. Wood, D. L. *et al. Science* **192**, 896–898 (1976).
9. Borden, J. H., Chong, L., McLean, J. A., Slessor, K. N. & Mori, K. *Science* **192**, 894–896 (1976).
10. Vité, J. P., Klimetzek, D., Loskant, G., Hedden, R. & Mori, K. *Naturwissenschaften* **63**, 582–583 (1976).
11. Vité, J. P., Hedden, R. & Mori, K. *Naturwissenschaften* **63**, 43 (1976).
12. Hedden R., Vité, J. P. & Mori, K. *Nature* **261**, 696–697 (1976).

Acetylcholine receptors in the oocyte membrane

In vertebrates, fully differentiated cells such as skeletal muscle and some nerve cells are highly sensitive to acetylcholine (ACh), due to the presence of acetylcholine receptors in their surface membranes. In muscle fibres, the ACh-sensitivity is known to appear early during differentiation^{1–3}, but relatively little is known about the chemical sensitivity of the membrane in the undifferentiated cell. In particular, it would be interesting to know if spermatozoa and oocytes are already sensitive to ACh and other neurotransmitter substances. To examine this question, toad (*Xenopus laevis*) oocytes were taken from the ovary, kept in frog's Ringer or Merriam⁴ solution, and studied at room temperature, using conventional electrophysiological techniques.

The oocytes had resting potentials of –30 to –80 mV, and their input resistance varied between 200 kΩ and over 3 MΩ. The membrane potential frequently showed transient spontaneous depolarisations a few mV in amplitude. When the oocytes were perfused with a solution containing ACh the membrane was depolarised as illustrated in Fig. 1a. This depolarisation was dose-dependent, but varied greatly among different oocytes, with some responding to as little as 10^{-8} M, while oocytes from other animals failed to respond to 10^{-3} M. Other cholinergic drugs such as carbachol, muscarine, arecholine and choline also depolarised the oocytes. The most usual response to ACh was a membrane depolarisation but frequently this was followed, or preceded, by a hyperpolarisation; and in some oocytes, only a hyperpolarising response was seen. Both types of response

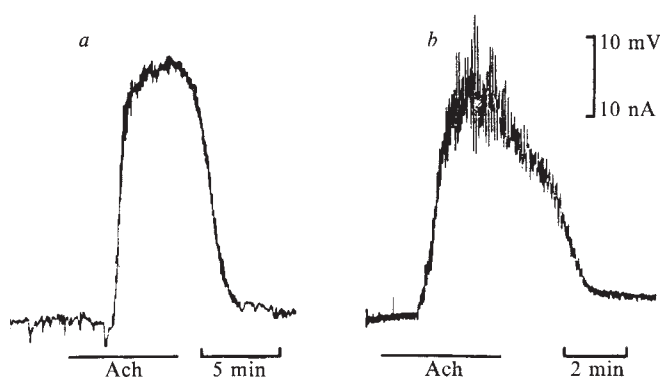


Fig. 1 Effect of acetylcholine (ACh) on a *Xenopus laevis* oocyte. *a*, Change in membrane potential induced by bath application of 10^{-5} M ACh. The bar indicates the time during which ACh was perfused. Resting potential -63 mV. *b*, Membrane current induced by perfusion of 10^{-5} M ACh. Same oocyte as in *a* but under voltage-clamp. Holding potential -53 mV. Oocyte pre-treated with collagenase to remove follicular cells.

'desensitised' during maintained application of ACh. To determine if the ACh was acting directly on the oocyte membrane, and not through an action on the follicular cells which normally envelop the oocytes, these were treated with collagenase which is known⁵ to remove the follicular cells. Such treatment sometimes reduced or even abolished the effect of ACh, but many treated oocytes responded normally to ACh and a subsequent morphological study showed that the follicular cells had actually been removed. Thus, it is clear that ACh is acting directly on the oocyte.

The depolarisation induced by ACh was accompanied by a fall in membrane resistance (Fig. 2), and an inward flux of current which could be measured under voltage-clamp conditions (Fig. 1*b*). The membrane potential at which the ACh-induced current reverses in direction was about -25 mV, and similar values were obtained from the cross-over point of the current-voltage relations before and during the application of ACh (Fig. 2) or from the plateau level of depolarisation evoked by high doses of ACh (Fig. 1*a*).

In other systems, ACh is known to alter membrane permeability to one or more of the following ions: Na^+ , K^+ , Cl^- and possibly Ca^{2+} (ref. 6). ACh was still able to depolarise the oocytes when Ca^{2+} was removed from the

external fluid, when Tris, Ca^{2+} or Mg^{2+} were completely substituted for Na^+ , or when all the Cl^- was replaced by SO_4^{2-} . In the latter case, the response to ACh was enhanced and the membrane potential during ACh-action even reversed in sign, becoming inside positive. The principal conclusion drawn from these experiments is that ACh increases the permeability of the oocyte membrane, mainly to Cl^- ions.

Some substances which are known to block the effects of ACh in other systems were tested to see if they antagonised the action of ACh on the oocyte. Curare (up to 10^{-4} M) was ineffective, as was also α -bungarotoxin (10^{-6} g ml $^{-1}$) which did not block the response to ACh even after the oocytes had been incubated in the toxin for 48 h. Tetrodotoxin (10^{-6} M), which blocks action potentials in some cells, did not abolish the spontaneous membrane depolarisations or the effect of ACh. On the other hand, atropine (10^{-7} to 10^{-4} M) blocked the action of ACh on the oocytes in a reversible way.

We have made a brief survey of the effect of some neurotransmitters on the oocytes, by perfusing the drugs at concentrations ranging from 10^{-6} to 10^{-3} M. Glycine, GABA, *L*-glutamate and histamine had no obvious effect on the membrane potential of collagenase-treated, or untreated oocytes. Adrenaline, 5-hydroxytryptamine and dopamine evoked a membrane hyperpolarisation accompanied by a decrease in membrane resistance (Fig. 3). As in the case of ACh, the sensitivity of the oocytes to the amines varied considerably from batch to batch. Some oocytes were sensitive to both ACh and dopamine, in which case atropine blocked only the response to ACh. Thus, ACh and dopamine seem to act on different membrane receptors, and evoke different permeability changes.

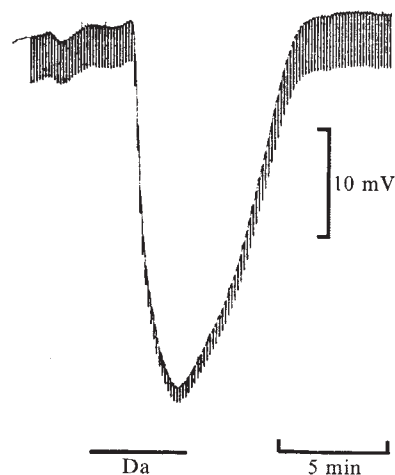


Fig. 3 Hyperpolarisation of oocyte membrane potential induced by dopamine (10^{-6} M). Membrane resistance decreased during dopamine action, as evidenced by the decrease in the potential change caused by the series of brief hyperpolarising pulses. Oocyte not pre-treated with collagenase. Resting potential, -41 mV.

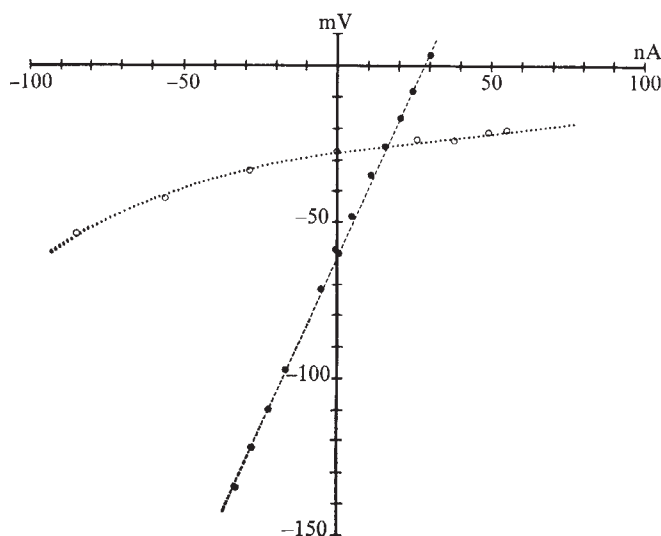


Fig. 2 Current-voltage relation of the oocyte membrane in resting state (full circles) and during depolarisation induced by 10^{-5} M ACh ($\circ$). Membrane conductance increased about 18-fold during peak ACh-action. Collagenase-treated oocyte.

During bath application of ACh (Fig. 1), it was noticed that the response began well after the 'dead-time' of the perfusion system (about 1 min). To obtain a better indication of the time lag between the application of ACh and the onset of ACh-action, we applied the ACh ionophoretically to a small patch of the oocyte's surface membrane. Using this method on muscle fibres it has been possible⁷ to detect a response to ACh a small fraction of a millisecond after the release of ACh. In contrast, the responses in the oocyte always took many seconds to develop (Fig. 4) and consisted of a series of oscillations of membrane potential. Similar

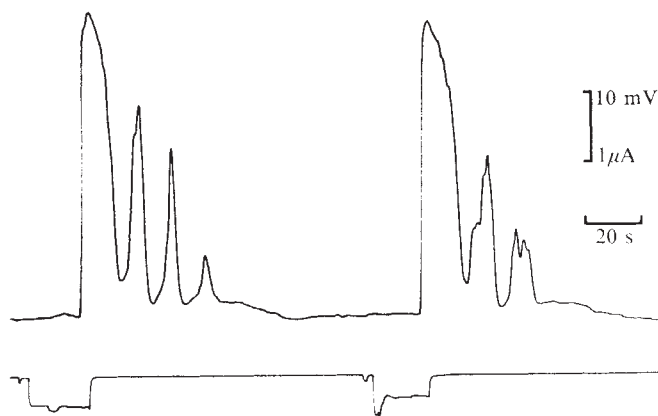


Fig. 4 Oscillations of membrane potential induced by iontophoretic application of ACh to an oocyte pre-treated with collagenase. Upper trace: membrane potential. Lower trace: ionophoretic current used to release ACh. The small deflexion preceding each pulse gives a measure of the backing current used to retain the ACh in the pipette. Resting membrane potential, -62 mV.

oscillations in current were seen when the oocyte was in voltage-clamp (cf. Fig. 1b).

Micro-iontophoresis of ACh also allowed us to examine the distribution of ACh-sensitivity over the oocyte's surface. There was a large variation in the sensitivity of different spots, but nevertheless it was clear that both the pigmented and non-pigmented surfaces of the oocyte were sensitive to ACh. Furthermore, it seems that the ACh-receptors are only activated by ACh from the outside, because no depolarisation was obtained when the ACh was applied intracellularly.

Thus, it seems that the oocytes synthesise, and incorporate into their surface membrane, receptors which are activated by neurotransmitters and related substances. The functional significance of these receptors in the oocyte is not known, although it has been suggested that ACh and some monoamines may act as intracellular regulators of cell division^{8,9}.

Our experiments indicate that the drug-receptor combinations open channels through which ions flow across the oocyte membrane. The long delay between the application of ACh and the onset of the increase in membrane permeability indicates that the receptor-channel coupling is not as fast as in skeletal muscle. Perhaps a number of receptors have to interact before they are able to open the channel; or it may be that in the oocyte the opening of the channel is not triggered directly by the receptor, but indirectly through the production of a substance, perhaps within the cell, and its accumulation near the membrane.

K.K. was supported by USPHS (NS-12275), J.S. by C.N.R.S. of France, and R.M. by MRC.

K. KUSANO

R. MILEDI

J. STINNAKRE

Department of Biophysics,
University College London,
Gower Street,
London, UK

Received 27 July; accepted 25 October 1977.

1. Diamond, J. & Miledi, R. *J. Physiol., Lond.* **162**, 393–408 (1962).
2. Ritchie, A. K. & Fambrough, D. M. *J. gen. Physiol.* **65**, 327–356 (1975).
3. Blackshaw, S. & Warner, A. *Nature* **262**, 217–218 (1976).
4. Merriam, R. W. *Expl Cell Res.* **68**, 81 (1971).
5. Moreau, M., Guerrier, P., & Dorée, M. *C. r. hebdom. Séanc. Acad. Sci. Paris* **D282**, 1309–1312 (1976).
6. Ginsborg, B. L. *Biochim. Biophys. Acta* **300**, 289–317 (1973).
7. Katz, B. & Miledi, R. *Proc. R. Soc. Lond.* **B 161**, 483–495 (1965).
8. Reshetnikova, N. A. *Zh. Evol. Biochim. I. Fiziol.* **6**, 19–24 (1970).
9. Buznikov, G. A. *Ontogenez* **2**, 5–13 (1971).

Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones

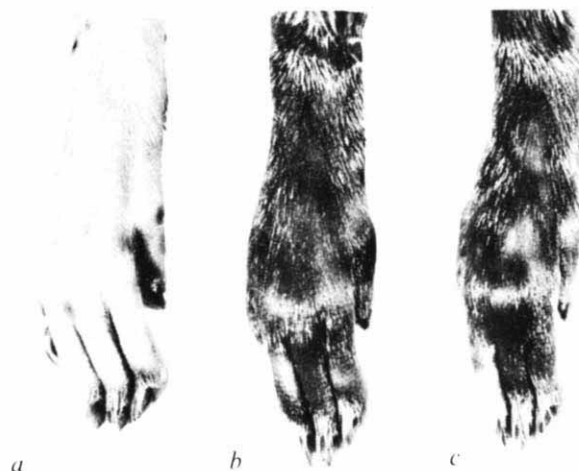
PHARMACOLOGICALLY evoked selective degeneration of neurones with a specific function or functioning with known transmitter substances, has been reported only in the monoaminergic neurone systems^{1,2}. We report here that selective degeneration of neurones with a highly specific function can be produced by chemical agents in the somatosensory system as well. Capsaicin given to newborn rats induces selective degeneration of a distinct population of primary sensory neurones involved in mediation of chemogenic pain.

Capsaicin—after an initial violent stimulation—renders sensory nerve endings in the skin and mucous membranes of different species insensitive to chemical pain stimuli for a long time^{3,4}. In addition, in the rat, marked systemic desensitisation greatly inhibits the neurogenic inflammation induced either by pain-producing chemical irritants, or by antidromic electrical stimulation of sensory nerves^{5,6}. The neurohumoral substance which increases vascular permeability is released from the sensory nerve endings which transmit chemogenic pain^{7–8}. Thus the degree of the increase in vascular permeability can be used as a tool to study the functional condition of primary sensory neurones involved in the transmission of pain induced by chemical stimuli. The desensitising effect of capsaicin has been analysed in detail in the adult rat, and it has been established that the functional impairment ensues at the level of the primary sensory neurone^{6,9,10} without causing its degeneration¹¹.

Newborn rats of the CFY strain were given a moderate subcutaneous dose of 50 mg kg^{-1} capsaicin on day 2 of life. Animals receiving a similar amount of the solvent used (10% ethanol, 10% Tween 80 in isotonic saline) served as controls. Adult rats, aged 3–4 months, were similarly treated. Neurogenic inflammation was brought about by painting the skin of one of the hind paws with 5% mustard oil in liquid paraffin or with xylene^{5,6} after intravenous administration of 50 mg kg^{-1} Evans Blue dye. Twenty minutes afterwards, the animals were bled and the amount of exuded dye was extracted from the skin with methanol containing 1% Suramin and determined photometrically¹².

Table 1 shows the results of a series of experiments

Fig. 1 Effect of 5% mustard oil on the skin of the paw of a rat pretreated *a*, neonatally and *b*, as an adult with 50 mg kg^{-1} capsaicin 9 months before the experiment; *c*, normal control. Evans Blue dose was 50 mg kg^{-1} intravenously.



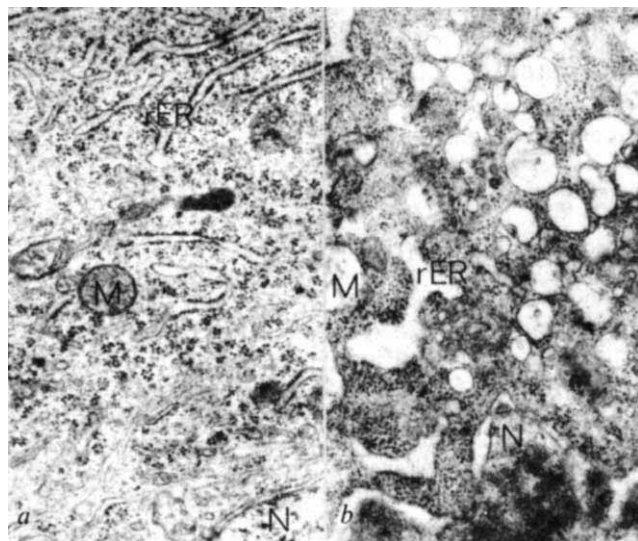


Fig. 2 Electron micrograph of B-type neurones of cervical sensory ganglia of 2-d-old rats. *a*, Control; *b*, 30 min after capsaicin treatment. As a consequence of capsaicin treatment, the fine structure of the primary sensory neurone is severely impaired; note swollen mitochondria, disorganisation of cristae, dilation of the perinuclear cisterna, as well as that of the cisternae of the rough endoplasmic reticulum. The electron density of the cytoplasm much greater than that of the control. N, Nucleus; M, mitochondrion; rER, cisterna of the rough endoplasmic reticulum. Magnification $\times 16,000$.

carried out on rats pretreated, as neonates or as adults, with capsaicin 2–8 months before the experiment. The amount of dye exuded from rats pretreated as adults did not differ considerably from that of the controls. Neonatal pretreatment, however, resulted in an almost complete blockade of the neurogenic inflammatory response. Figure 1 shows the results of a typical experiment, where pretreatment was 50 mg kg^{-1} capsaicin 9 months before eliciting neurogenic inflammation in neonatal and adult rats. The paw of the control rat turned blue in response to 5% mustard oil solution, indicating the intense increase in the vascular permeability. A comparable dye exudation also ensued in the skin of a rat pretreated as an adult. Neurogenic inflammation could not, however, be elicited in the paw of the neonatally-pretreated rat. The same results were obtained if xylene was used as the irritant. Chemical sensitivity in the eye, as tested by zingerone, the pungent pain-producing substance in ginger⁵, was practically abolished in these animals, but they showed no reduction in sensitivity to physical, for example, mechanical, stimuli. Neonatal capsaicin pretreatment evidently impairs the function of the chemosensitive primary sensory neurones irreversibly.

For histological investigations, animals were perfused with a buffered solution of 2% glutaraldehyde and 4% formaldehyde by way of the left ventricle, at different times

after neonatal capsaicin pretreatment. The Gasserian ganglion, cervical sensory ganglia and attached dorsal roots and the cervical spinal cord were processed for standard electron microscopic examination. In agreement with previous reports, the two principal types of sensory ganglion cells, the large type A neurones and the small type B neurones¹³ could be distinguished even at the very early stage of postnatal development¹⁴. Capsaicin injected to 2-d-old rats exclusively damaged some of the B-type neurones. Only 30 min after administration of capsaicin, these neurones showed severe fine structural alterations (Fig. 2). The ultrastructure of the type A neurones and other cellular elements of sensory ganglia was unimpaired after capsaicin treatment. Similarly, the fine structure of all cellular elements of sensory ganglia obtained from control animals appeared normal.

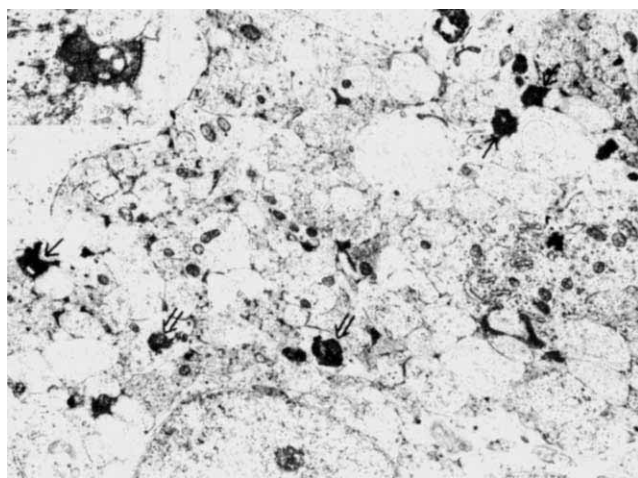


Fig. 3 Electron micrograph showing degenerated axons and axon terminals (arrows) partially engulfed by glial processes (double arrows), in Rexed's lamina II of the cervical spinal cord 8 h after capsaicin administration to a 2-d-old animal. Inset: Degenerated axon terminal still in synaptic contact with a postsynaptic dendrite. Magnification: $\times 5,920$, inset $\times 13,500$.

Light microscopy of 3–6- μm thick, *p*-phenylenediamine-stained¹⁵ Araldite sections of the cervical spinal cord of neonatally-treated rats which had been killed 8 h after treatment, revealed degeneration of axon terminals in the head of the dorsal horn, mainly in the laminae I and II of Rexed. Electron microscopy confirmed this finding by showing the presence of a considerable number of degenerating axons and axon terminals in this area (Fig. 3). After survival for 15 h, most of the degenerated axon terminals were engulfed by glial processes. The degenerating axon terminals are, in all probability, the central processes of the impaired B-type sensory ganglion cells, since 4 h after capsaicin treatment many unmyelinated nerve fibres in the

Table 1 Evans Blue dye exudation elicited by 5% mustard oil in the paw skin of rats pretreated with capsaicin 2–8 months before the experiment

Rats (No.)	Pretreatment	Excess dye (μg) ($\pm \text{SE}$)	Inhibition (%)
17	—	23.75 ± 4.03	
17	50 mg kg^{-1} capsaicin (neonatally)	1.48 ± 0.5	93.8
10	—	27.78 ± 6.18	
10	50 mg kg^{-1} capsaicin (adult)	19.05 ± 6.45	31.5

The excess dye values were obtained by subtracting the dye content of the control skin from that of the treated skin. Evans Blue dose 50 mg kg^{-1} intravenously.

dorsal root underwent degeneration. In addition, experiments now in progress show, that one month after neonatal capsaicin treatment there is about a 70% reduction in the number of unmyelinated fibres of the saphenous nerve.

Axonal degeneration in the dorsal horn was accompanied by the appearance of many glial cells, localised mainly in the laminae I and II of Rexed. In stained sections, the cytoplasm of these cells contained intensely-stained granules and they were also characterised by a very marked acid phosphatase enzyme activity. In the electron microscope sections, numerous lamellar bodies and osmiophilic debris were observed in the cytoplasm of these cells. It therefore seems that these cells are phagocytosing glial cells, ingesting degenerated neuronal elements.

The findings presented here clearly show that neonatal capsaicin treatment results in a completely irreversible impairment of the function of chemosensitive primary sensory neurones. As a consequence, painful sensory irritants, the effect of which depends on intact chemosensitive nerve endings, do not evoke neurogenic inflammation in neonatally-treated animals. The morphological basis for this phenomenon is provided by the degeneration of a distinct population of primary sensory neurones. In adult rats treated with capsaicin, the effect of neurogenic irritants gradually reappeared, showing that the function of chemosensitive pain nerve endings, at least partially, returned. This latter finding is consistent with previous results, which stated that capsaicin treatment does not induce degeneration of primary sensory neurones in the adult rat¹¹.

Our present results may open new possibilities in the study of the structure and function of chemosensitive nerve endings, as well as in mapping of their central neuronal connections. These findings may also promote investigations to elucidate, for example, immunohistochemically, which of the putative transmitters¹⁶ may be involved in mediation of chemogenic pain.

G. JANCÓS
ELISABETH KIRALY

Department of Anatomy,

AURELIA JANCÓS-GÁBOR

Department of Physiology,
University Medical School,
Szeged, Hungary

Received 21 June; accepted 25 October 1977.

- Kostrzewa, R. M. & Jacobowitz, D. M. *Pharmac. Rev.* **26**, 199–288 (1974).
- Baumgarten, H. G., Björklund, A., Nobin, A., Rosengren, E. & Schlossberger, H. G. *Acta physiol. scand., Suppl.* **429**, 7–29 (1975).
- Jancsó, N. *Bull. Millard Fillmore Hosp. Buffalo N.Y.* **7**, 53–77 (1960).
- Jancsó, N. & Jancsó-Gábor, A. *Arch. exp. Path. Pharmac.* **236**, 142–145 (1959).
- Jancsó, N. *Proc. 3rd Int. Pharmac. Mtg.* 1966. *Pharmacology of Pain* **9**, 33–55 (Pergamon, Oxford, New York, 1968).
- Jancsó, N., Jancsó-Gábor, A. & Szolcsányi, J. *Br. J. Pharmac.* **31**, 138–151 (1967).
- Jancsó-Gábor, A. & Szolcsányi, J. *J. dent. Res.* **51**, 264–269 (1972).
- Garcia Leme, J. & Hamamura, L. *Br. J. Pharmac.* **51**, 383–389 (1974).
- Pörzsász, J. & Jancsó, N. *Acta physiol. Acad. Sci. Hung.* **16**, 299–306 (1959).
- Jancsó, G. & Knyihár, E. *Neurobiology* **5**, 42–43 (1975).
- Jóó, F., Szolcsányi, J. & Jancsó-Gábor, A. *Life Sci.* **8**, 621–626 (1969).
- Jancsó-Gábor, A., Szolcsányi, J. & Jancsó, N. *J. Pharm. Pharmac.* **19**, 486–487 (1967).
- Andres, K. H. Z. *Zellforsch.* **55**, 1–48 (1961).
- Yamadori, T. *Acta Anat. Nippon.* **45**, 191–205 (1970).
- Holländer, H. & Vaaland, J. L. *Brain Res.* **10**, 120–126 (1968).
- Hökfelt, T. *et al. Neuroscience* **1**, 131–136 (1976).

D- α -Aminoadipate as a selective antagonist of amino acid-induced and synaptic excitation of mammalian spinal neurones

THE identification of either L-glutamate or L-aspartate as excitatory transmitters in the mammalian central nervous system would be facilitated by the discovery of specific antagonists of amino acid-induced and synaptic excitation. Hall *et al.* have suggested that D- α -aminoadipate may be an amino acid antagonist¹. They based their suggestion on the

depressant effect of the DL form on spontaneous and amino acid-induced excitation of rat thalamic neurones, the L form being weakly excitatory. We have shown that related long-chain amino acids depress synaptic excitation of spinal neurones and exert a differential depressant action on chemically induced excitation of these cells². We therefore tested the actions of both D- and DL- α -aminoadipate on synaptically evoked excitation and on excitations induced by acetylcholine, L-glutamate, L-aspartate, kainate and N-methyl-D-aspartate (NMDA) on spinal neurones. These latter two amino acids have been suggested to act on 'glutamate-preferring' and 'aspartate-preferring' receptors, respectively^{3,4}. The results presented here indicate that D- α -aminoadipate selectively antagonises NMDA- and L-aspartate-induced responses of dorsal horn interneurones and Renshaw cells and depresses non-cholinergic synaptic excitation of these cells evoked by dorsal root stimulation, whereas cholinergic excitation of Renshaw cells evoked by iontophoretic acetylcholine or ventral root stimulation is not depressed by this agent.

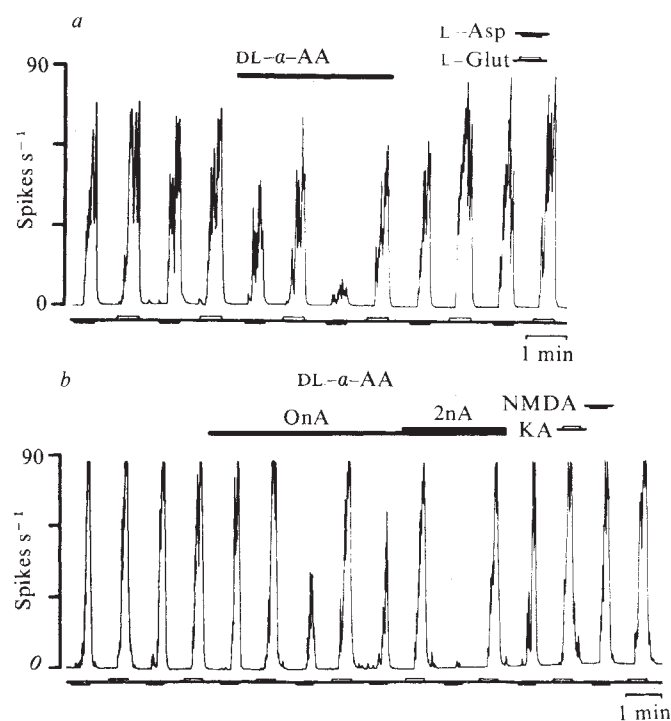


Fig. 1 Selective effects of DL- α -aminoadipate (DL-AA) on amino acid-induced excitation of a dorsal horn interneurone in the mouse spinal cord. *a*, DL-AA (6 nA), ejected for the period indicated by the upper bar, almost abolished excitation produced by L-aspartate (30 nA) and depressed responses of the cell to L-glutamate (38 nA) to a lesser extent. *b*, Responses of the same cell to NMDA (24 nA) were diminished by removal of the DL-AA retaining current and abolished by 2 nA of the agent, while responses to kainate (5 nA) were unaffected.

We used spinal interneurones of the mouse and spinal interneurones and Renshaw cells of the cat. Mice (C3H strain) were anaesthetised with pentobarbitone sodium (40 mg per kg body weight) given periodically through an intraperitoneal (i.p.) cannula as required. After i.p. administration of gallamine triethiodide (50 mg per kg), mice were ventilated artificially through a tracheal cannula at a respiratory rate of 166 min⁻¹. They rested on a thermistor-controlled heating pad (37–38 °C). (Further details are given in ref. 5.) Cats were anaesthetised with pentobarbitone sodium (35 mg per kg i.p. initially, supplemented by 5 mg per kg i.v. when necessary). The surgical procedure and methods of neurone identification were as previously described⁶. Conventional iontophoretic techniques were used in both series of experiments. Extracellular recordings

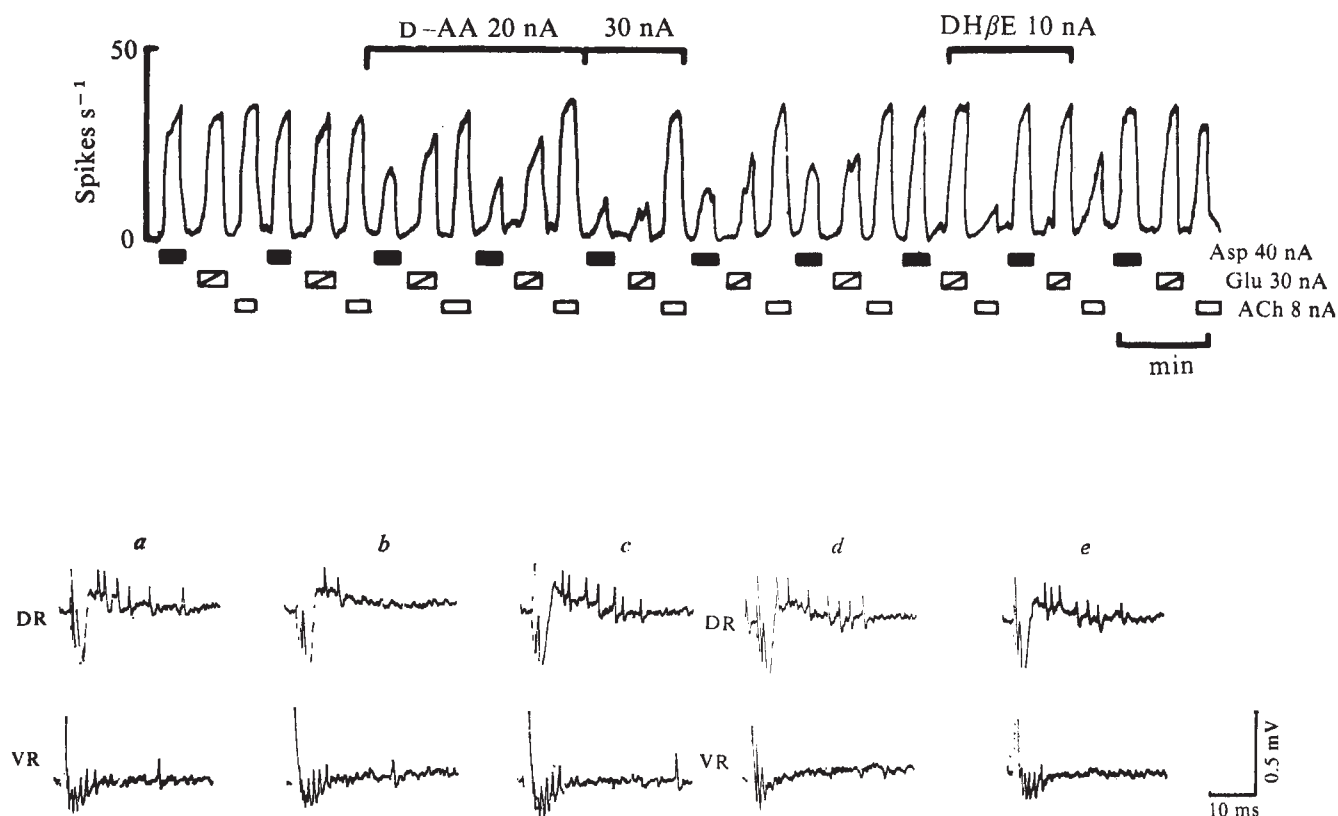


Fig. 2 Selective antagonist actions of D- α -aminoadipate (D-AA) and dihydro- β -erythroidine (DH β E) on chemically- and synaptically-induced firing of a cat Renshaw cell. The ratemeter record at the top of the figure illustrates the selective depression of L-glutamate (glu 30 nA) and L-aspartate (asp 40 nA)-induced excitation by D-AA 20–30 nA, and of acetylcholine (ACh 8 nA)-induced excitation by DH β E (10 nA). Records *a–e* are representative oscilloscope sweeps of the synaptic responses of the same Renshaw cell to a constant submaximal dorsal root (DR) and ventral root (VR) stimulus. *a* was taken during the control period, *b* during the ejection of D-AA 90 nA for 20 min, *c* 2 min after terminating the ejection of D-AA, *d* during the ejection of DH β E 50 nA for 2 min, and *e* 3 min after terminating the DH β E ejection. The mean number of spikes in 15 responses $\pm$ s.e.m. were, for upper and lower traces respectively, *a*, 5.8 ± 0.7 and 4.1 ± 0.2 ; *b*, 1.8 ± 0.1 and 4.7 ± 0.3 ; *c*, 5.3 ± 0.7 and 4.8 ± 0.2 ; *d*, 4.9 ± 0.6 and 2.3 ± 0.3 ; *e*, 4.7 ± 0.8 and 3.6 ± 0.3 . D-AA reversibly reduced the DR responses but not the VR responses (*b*), while DH β E reversibly reduced the VR responses but not the DR responses (*d*). These specific depressant effects of D-AA and DH β E are highly significant statistically ($P < 0.001$) in both cases, Student's *t* test).

were made with the 4 M NaCl-containing centre barrel of seven-barrel micropipettes, the other barrels containing solutions of agonists and antagonists as follows: sodium NMDA, 50 mM in 100 mM NaCl, pH 7; sodium kainate, 20 mM in 130 mM NaCl, pH 7; sodium L-glutamate and sodium L-aspartate, each 200 mM (mouse) or 500 mM (cat), pH 7; sodium D- and sodium DL- α -aminoadipate (Sigma) each 200 mM, pH 7; acetylcholine chloride (500 mM). D- α -Aminoadipic acid ($[\alpha]_D^{23.5} = -0.7$ in 6 N HCl) was isolated from the racemic mixture by fractional crystallisation of the D-lysine salt, and recovery of the free acid by ion exchange chromatography.

Both D- and DL- α -aminoadipate selectively depressed NMDA- and L-aspartate-induced excitation of dorsal horn interneurons in the mouse spinal cord. The cell illustrated in Fig. 1 was particularly sensitive to the antagonist and an ejection current of 2 nA was sufficient to abolish the excitation produced by NMDA. There was little or no effect on excitation evoked by kainate (Fig. 1*b*). A slightly higher antagonist-ejecting current (6 nA) almost abolished L-aspartate-induced excitation of the same cell, and had considerably less effect on L-glutamate-induced excitation (Fig. 1*a*). Recovery of the responses occurred rapidly after termination of the antagonist administration. Similar effects were seen on all 14 cells treated with either D- α -aminoadipate (11 ± 2 nA, 7 cells) or DL- α -aminoadipate (17 ± 6 nA, 7 cells). Both forms of α -aminoadipate also depressed the synaptic excitation of these cells evoked by dorsal root stimulation. This effect was seen on 8 of 10 cells tested with the D form (20–45 nA) and on all four cells tested with the DL form (5–40 nA).

When tested on cat Renshaw cells, both DL- α -aminoadipate (39 ± 12 nA, six cells) and D- α -aminoadipate (16 ± 4 nA, 10 cells) had a similar differential depressant effect on excitation induced by NMDA and kainate to that observed on mouse dorsal horn interneurons. L-Aspartate-induced excitation was also more sensitive than L-glutamate-induced excitation of these cells, though this differential sensitivity tended to diminish with increased ejection currents of antagonist (Fig. 2). Acetylcholine-induced excitation of the same Renshaw cells was virtually unaffected by either form of the agent. Of 16 Renshaw cells excited by acetylcholine, 13 were unaffected, 1 depressed and 2 slightly enhanced by DL- or D- α -aminoadipate (6 and 10 cells, respectively).

Renshaw cells may be activated synaptically by both cholinergic and non-cholinergic pathways, and in the present experiments these different synaptic inputs were differentiated by the use of the nicotinic antagonist dihydro- β -erythroidine (DH β E). D- α -Aminoadipate (40–100 nA for 4–20 min) had no effect on the DH β E-sensitive excitation of 11/11 quiescent Renshaw cells evoked by ventral root stimulation. In contrast, DH β E-resistant excitation evoked by dorsal root stimulation was markedly depressed by D- α -aminoadipate (20–90 nA for 3–5 min) on 5/5 of these cells (Fig. 2). The firing of a further 7 spontaneously active Renshaw cells was also depressed by either D- or DL- α -aminoadipate (10–80 nA).

Experiments on the isolated frog spinal cord have indicated that the whole of the depressant activity of DL- α -aminoadipate is due to the D isomer (our unpublished observations). In other experiments we have shown that D- α -aminoadipate has little or no effect on responses of the

isolated rat spinal cord to carbachol, substance P or nor-adrenaline and that the agent does not affect transmission in the rat superior cervical ganglion. These findings, together with the specificity shown by the agent in depressing chemically-induced and synaptically-evoked excitations in the present work, strongly suggest that D- α -amino adipate acts by a specific antagonism of amino acid-mediated synaptic excitation in the spinal cord. It follows that an acidic amino acid is probably released synaptically on to mouse dorsal horn interneurons and cat Renshaw cells in response to dorsal root stimulation. Since the actions of kainate and L-glutamate are less sensitive to the antagonist than those of NMDA and L-aspartate, our results support previous evidence^{4,8,9} that the transmitter mediating such synaptic excitation may be L-aspartate.

We thank Mr D. J. Oakes, Mrs Ann Duncan and Miss C. Rickets for technical assistance. This work was supported by the MRC.

T. J. BISCOE
R. H. EVANS
A. A. FRANCIS
M. R. MARTIN
J. C. WATKINS

Departments of Physiology and Pharmacology,
The Medical School,
University of Bristol, Bristol, UK

J. DAVIES
A. DRAY

Department of Pharmacology,
The School of Pharmacy,
University of London,
Brunswick Square, London WC1, UK

Received 22 August; accepted 22 October 1977.

- Hall, J. G., McLennan, H. & Wheal, H. V. *J. Physiol., Lond.* 272, 52–53P (1977).
- Biscoe, T. J. *et al. Eur. J. Pharmac.* 45, 315–316 (1977).
- Johnston, G. A. R., Curtis, D. R., Davies, J. & McCulloch, R. M. *Nature* 248, 804–805 (1974).
- McCulloch, R. M., Johnston, G. A. R., Game, C. J. A. & Curtis, D. R. *Expl Brain Res.* 21, 515–518 (1974).
- Biscoe, T. J., Headley, P. M., Martin, M. R. & Sterling, C. A. *J. neurol. Sci.* 31, 51–61 (1977).
- Davies, J. & Watkins, J. C. *Brain Res.* 130, 364–368 (1977).
- Curtis, D. R. & Ryall, R. W. *Expl Brain Res.* 2, 81–96 (1966).
- Davidoff, R. A., Graham, L. T., Shank, R. P., Werman, R. & Aprison, M. H. *J. Neurochem.* 14, 1025–1031 (1967).
- Duggan, A. W. *Expl Brain Res.* 19, 522–528 (1974).

Axon conduction velocity modified by reinnervation of mammalian muscle

WHEN Buller *et al.*¹ observed that cross reinnervation of fast- and slow-twitch muscles could reverse their mechanical properties, the operations had been performed in the hope of finding changes in reflex connections. The changes in muscles were of great interest and have dominated this

field of research until recently, when Kuno *et al.* investigated some changes in motoneurone properties following axotomy and reinnervation. Retrograde changes of some electrical properties of the soma were observed which were reversed when the nerve re-established connections with the muscle. We report here some experiments on motor units in normal and reinnervated muscles, in which we have found that the conduction velocities of regenerated axons depend on the nature of the muscle which they reinnervate.

The initial operations were carried out on young adult cats of 1.5–2 kg body weight. Under anaesthesia, the nerves to flexor digitorum longus (FDL) and soleus muscles (fast- and slow-twitch respectively) were divided between fine ligatures. Each central end was reunited either to its own distal stump (self reinnervation) or to that of the other nerve (cross reinnervation).

For the final experiment either FDL or soleus was prepared for isometric recording and ventral roots were exposed by laminectomy. Functionally single axons were isolated by splitting the ventral roots with fine forceps as described elsewhere^{3,4}. Only axons producing a response in the muscle under test were examined, but some of these would have branched to innervate the other muscle.

Axon conduction velocity was estimated from the latency of the antidromic action potential and the inter-electrode conduction distance (168–198 mm) measured *in situ* proximal to the neuroma. The stimulus was set at twice threshold for a 0.2-ms rectangular pulse. Axonal conduction velocity increases during regeneration but this could not affect the interpretation of our results since there were no significant differences between the post-operative survival periods of the self and cross re-innervated groups (Table 1b).

The axonal conduction velocities recorded in these experiments are displayed as the histograms of Fig. 1. In each panel, the open columns indicate axons which had originally innervated FDL muscles. In the control and self reinnervated groups these axons still innervated FDL muscle; in the cross reinnervated groups the 'FDL axons' innervated soleus muscle at the time of recording. In the same way, the hatched columns indicate soleus axons. A statistical analysis of the results is presented in Table 1a.

In normal muscles, FDL axons have a higher mean conduction velocity than those of soleus. After section and self reinnervation for 6 months, this difference persisted, although both groups of nerves conduct more slowly—at a mean velocity which was 83% of normal in FDL and 87% in soleus. This finding was anticipated, since slowing of conduction velocity proximal to the region of damage is well known.

In the FDL axons cross reinnervating soleus for 6 months, a reduction in conduction velocity to 83% of

Table 1 Statistical analyses of results of experimental reinnervation of muscle

Experimental group	Control	Self (6 month)	Cross (6 month)	Cross (2 yr)
<i>a</i> , Conduction velocity (m s ⁻¹)				
FDL	94.2 (205) ± 9.4	78.3 (24) * ± 10.1	78.5 (62) * ± 8.9	87.2 (115) *† ± 12.9
Soleus	67.5 (172) ± 8.0	58.7 (34) *† ± 14.1	71.9 (142) * ± 8.7	—
<i>b</i> , Reinnervation period (d)				
FDL	—	200 (5) ± 18	180 (5) ± 23	745 (5) ± 7
Soleus	—	215 (4) ± 20	198 (5) ± 15	—

The initial values are means; in parentheses are the number of axons (*a*) or of animals (*b*); ± s.d.

Significant differences between means * (comparison with corresponding control axons) and † (comparison with corresponding 6-month cross reinnervated axons, control group not indicated thus). In all these cases the differences by the *t*-test were significant at least at the 0.0001 level.

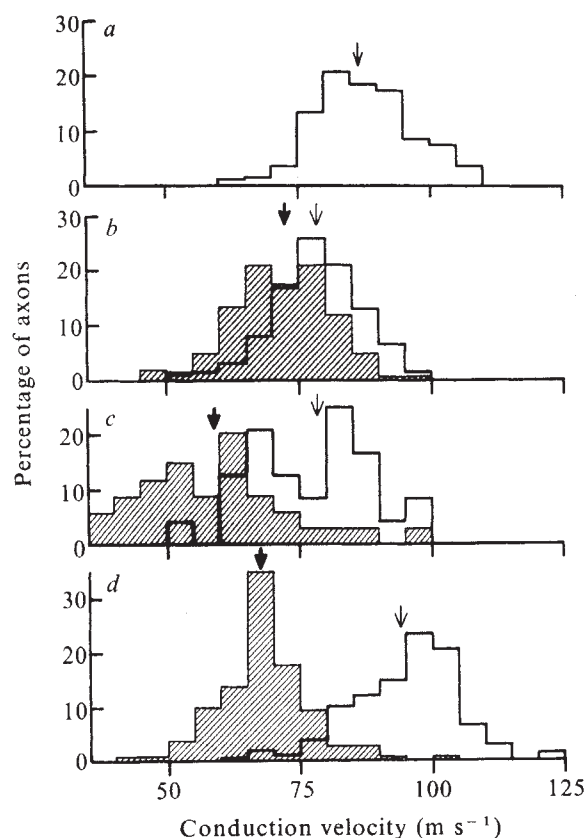


Fig. 1 Distributions of conduction velocities of FDL (open columns, light arrows) and soleus (hatched columns, dark arrows) axons in control cats and after reinnervation operations. Arrows indicate group means. *a*, Cross reinnervation, 2 yr; *b*, cross reinnervation, 6 months; *c*, self reinnervation, 6 months; *d*, control.

control was observed, which was very close to that in the corresponding self reinnervated group. We have followed cross reinnervated FDL axons for 2 yr and found that although recovery progressed over this longer period it was still not complete.

In contrast the mean conduction velocity of soleus axons cross reinnervating FDL muscle at 6 months was greater by 7% than that of normal soleus axons. The mean conduction velocity of the cross reinnervated soleus axons was 24% greater than that of the self reinnervated axons; the difference was highly significant ($t=6.9$, $P<10^{-6}$).

The present results seem to differ from those of Kuno *et al.*², who concluded that conduction velocities of soleus axons were restored to normal, both in self and in cross reinnervation, at 4–5 months. Their figures do, however, show more complete restoration following cross reinnervation and although this difference was not significant statistically (their numbers were smaller) it was in the same direction as ours and there may be no real discrepancy. Kuno *et al.*² did not examine cross reinnervated fast muscle nerves.

We conclude that the properties of reinnervating α -motoneurons, as expressed in the conduction velocity of their axons above the neuroma, are influenced by the nature of the muscle into which they grow. In particular, axons which normally innervate a slow-twitch muscle may have their conduction velocity increased by being made to innervate a fast-twitch muscle. Presumably, the anatomical basis of this would be an increase in axon diameter. Two general hypotheses are possible. One involves a direct influence from the muscle as has been suggested by Kuno *et al.*²; alternatively, the effect might involve a change in afferent information from a muscle receiving a foreign innervation with a different pattern

of activity. The second type of hypothesis could be extended more easily to explain why the effects are asymmetrical between fast and slow muscle.

J.B. and S.N.W. were supported by the Muscular Dystrophy Group of Great Britain; part of the work was supported by a grant from the Muscular Dystrophy Association of America.

D. M. LEWIS
J. BAGUST*
SANDRA N. WEBB†
R. A. WESTERMAN‡
H. J. FINOL§

Department of Physiology,
University of Bristol,
University Walk,
Bristol, UK

Received 14 July; accepted 4 November 1977.

Present addresses: * Department of Physiology, University of Southampton, Southampton. † Department of Pharmacology, University of Liverpool, Liverpool L69. ‡ Department of Physiology, Monash University, Australia. § Departamento de Biología Celular, Universidad Central, Caracas, Venezuela.

1. Buller, A. J., Eccles, J. C. & Eccles, R. M. *J. Physiol., Lond.* **150**, 417–439 (1960).
2. Kuno, M., Miyata, Y. & Munoz-Martinez, E. *J. Physiol., Lond.* **242**, 273–288 (1974).
3. Bagust, J., Knott, S., Lewis, D. M., Luck, J. C. & Westerman, R. A. *J. Physiol., Lond.* **231**, 87–104 (1973).
4. Bagust, J. & Lewis, D. M. *J. Physiol., Lond.* **237**, 91–102 (1974).

Birefringence signals and calcium transients in skeletal muscle

TWITCHES in skeletal muscle are preceded and accompanied by changes in optical properties of the muscle fibres^{1–11}. Measurements of birefringence give large signals which can be separated into two main components^{9–8}: the first begins on the falling phase of the action potential, and reaches a peak at the onset of tension development; this is followed by a late signal which is thought to be associated with development of tension by the contractile proteins^{2,3,6}. Since the early signal precedes tension development, it could provide an important tool for studying intervening steps in excitation–contraction coupling. This signal has recently been attributed to potential changes in the sarcoplasmic reticulum (SR) membrane, associated with Ca^{2+} release into the sarcoplasm^{6,10}. We have simultaneously recorded birefringence signals and changes in intracellular Ca^{2+} concentration, using arsenazo III, in frog muscle fibres. The results show that the onset of the early birefringence signal coincides with the rise in sarcoplasmic Ca^{2+} concentration, and that both can be abolished by injecting EGTA to chelate the sarcoplasmic Ca^{2+} . This suggests that the early birefringence signal arises from some process dependent on the rise in sarcoplasmic Ca^{2+} , and is not caused by events in the SR associated with the calcium release mechanism.

Experiments were performed on the cutaneous pectoris muscle of *Rana temporaria*, using a bathing solution of the following composition (in mmol l^{-1}): NaCl, 120; KCl, 2; CaCl_2 , 2; phosphate buffer (pH 7.2), 3. Intracellular Ca^{2+} changes were measured with arsenazo III as previously described^{12,13}. Briefly, the muscle was stretched sufficiently to block visible contraction, and a superficial fibre was impaled with two microelectrodes about 150 μm apart, for voltage recording and dye injection. Arsenazo III was injected by iontophoresis. The dye pipette also served to initiate action potentials by passing 1-ms depolarising current pulses or, in some experiments, to voltage-clamp a region of the fibre. White light was focused on a spot of 80 μm diameter between the pipettes, and the light transmitted through the fibre was diverted on to three photomultipliers. Calcium-dependent changes in absorption of arsenazo III were recorded by subtracting electrically

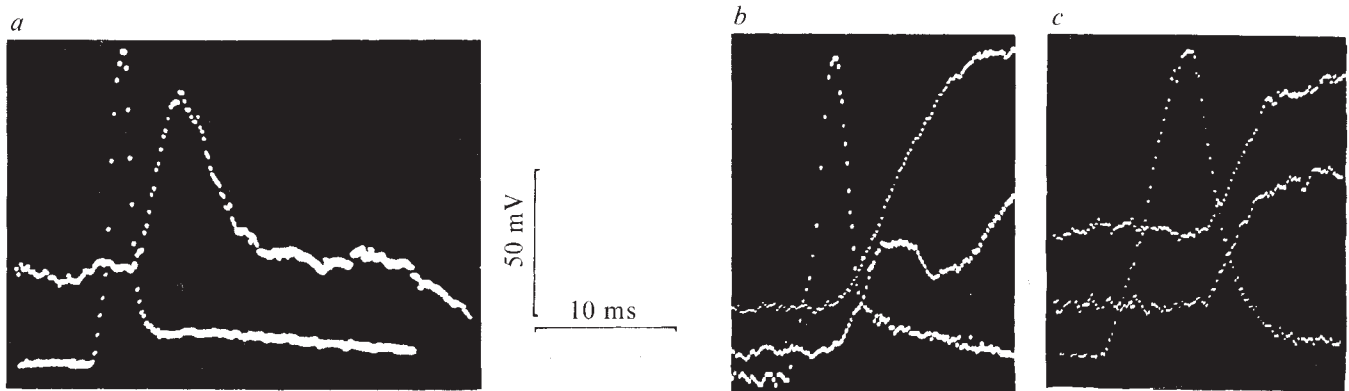


Fig. 1 Changes in birefringence and light absorption of arsenazo III following action potentials. Traces are signal averages of 32, 16 and 24 sweeps in *a*, *b* and *c*, respectively. In all panels, the bottom trace is the membrane potential; birefringence changes are shown in the upper trace in *a* and in the middle traces in *b* and *c*. Changes in sarcoplasmic Ca^{2+} concentration detected with arsenazo III are shown in the top traces in *b* and *c*. Both optical records were low-pass filtered with a time constant of 1 ms, and are expressed as the change in light transmitted through the fibre, divided by the resting transmission. Fractional changes in light transmission; 10^{-2} for the arsenazo III trace, and 2.5×10^{-3} for the birefringence. An upward deflection in both optical traces indicates a decrease in light intensity. Temperature: *a* and *b*, 10°C ; *c*, 4°C .

the measurements of transmitted light at wavelengths of 532 and 602 nm, obtained by using two photomultipliers and interference filters. Birefringence changes were recorded with the third photomultiplier and two crossed polarisers, once placed in front of the light source and the other in front of the photomultiplier. The plane of polarisation was at 45° to the longitudinal axis of the muscle fibres^{4,8}.

Preliminary experiments indicated that intracellular injection of arsenazo III did not affect the time course of the birefringence signals recorded following action potentials. The birefringence records show two components; an early

transient decrease in light intensity, followed by a signal with a similar time course to the movement artefact detected when one polariser was removed. This late component could appear as either an increase or a decrease in light intensity, depending critically on the positioning of the light spot. In a few fibres, the applied stretch sufficiently reduced the late signal to allow the early signal to be recorded with minimal distortion (Fig. 1*a*), but generally the early signal was obscured after its peak (Fig. 1*b* and *c*). At 10°C the early signal takes off within 2 ms of the foot of the action potential, and reaches a peak within 6 ms. The onset of the birefringence signal coincides with the onset of the calcium response as detected by the arsenazo III, and the latencies of both are affected to the same extent by changes in temperature (Fig. 1*b* and *c*).

Intracellular injection of EGTA provides a means of exploring the relationship between the events underlying these optical changes, since it reduces the increase in sarcoplasmic Ca^{2+} by binding the Ca^{2+} ions released by the SR (ref. 14). A micropipette containing 0.2 M EGTA (buffered to pH 7 with KOH) was inserted with its tip in the centre of the measuring light spot. A depolarising backing current (10 nA) was applied to the pipette while the control records were taken. To minimise movement artefacts resulting from contraction of distant regions of the fibre, action potentials were blocked with tetrodotoxin (10^{-6} g ml^{-1}); tetraethylammonium bromide (30 mM) was added to the medium and the membrane was voltage-clamped in the recording area. The fibre was stimulated by giving 5- or 10-ms depolarising pulses and EGTA was injected by

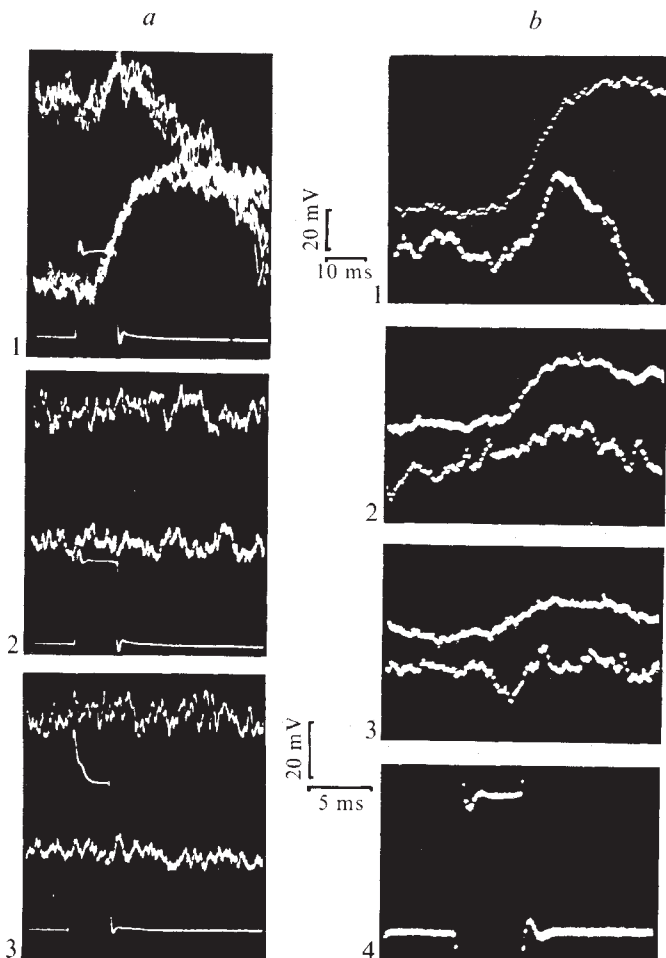


Fig. 2 Effect of EGTA on the birefringence signals and Ca^{2+} transients. The fibres were stimulated with 10-ms depolarising pulses in *a* and 5-ms pulses in *b*; tetrodotoxin (10^{-6} g ml^{-1}) and tetraethylammonium (30 mM) were present in the medium. *a* and *b* are records from two different fibres. *a*, Bottom trace is membrane potential, middle trace is arsenazo III responses and top trace shows birefringence changes. *a1* shows control responses (three superposed sweeps); *a2* and *a3* show the blockade of the optical signals following injection of EGTA for 8 min with an average current of 50 nA. Resting potential: 85 mV. *b*, Changes in dye absorption (upper trace in *b1*–*b3*) and in birefringence (lower trace in *b1*–*b3*) induced by depolarising pulses (shown in *b4*). Records are averages of 48 sweeps. EGTA was applied for 2 min at 40 nA between *b1* and *b2*, and for 2 min at 60 nA between *b2* and *b3*. Upper calibration bars apply to fibre *a*: horizontal, 10 ms; vertical, 20 mV, 2×10^{-3} (arsenazo III response) and 4×10^{-3} (birefringence changes). Lower calibration bars refer to fibre *b*: horizontal, 5 ms; vertical, 20 mV, 4×10^{-3} (arsenazo III) and 2×10^{-3} (birefringence). Temperature: *a*, 7°C ; *b*, 6°C .

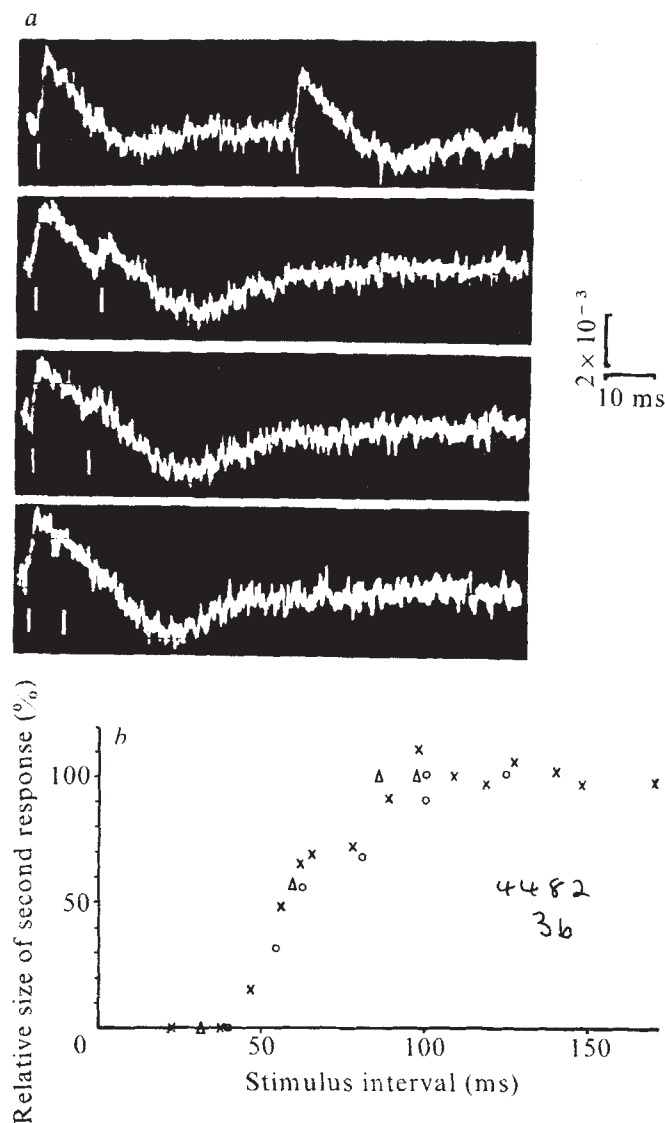


Fig. 3 Birefringence response to action potentials. *a*, Single sweeps showing four runs at stimulus intervals of 250, 65, 55 and 35 ms; vertical bars under the birefringence traces indicate the peak time of the action potentials; temperature, 4°C . *b*, Relative size of the birefringence signal elicited by the second pulse as a function of stimulus interval. Data from three different fibres; temperature, 4°C .

applying a small hyperpolarising current through the micro-electrode. The results are shown in Fig. 2. Blockade of the rise in free sarcoplasmic Ca^{2+} was accompanied by inhibition of both the early and the late birefringence signals (Fig. 2*a*); the intensity of the block could be graded by progressive increase in the amount of EGTA injected (Fig. 2*b*). In one fibre, a partial recovery of the birefringence and calcium signals was observed when recording was continued for about 30 min after injection. The most direct interpretation of these results is that the birefringence signals were abolished as a consequence of the reduction in size of the Ca^{2+} transient due to Ca^{2+} binding to the EGTA (ref. 14), but the possibility cannot be entirely ruled out that the EGTA additionally interfered in some way with the Ca^{2+} release mechanism.

Experiments with paired stimuli provide further evidence against the early birefringence signal being associated with the calcium release mechanism. When the interval between the two stimuli was less than 40 ms (at 4°C), no early birefringence change was detected following the second stimulus (Fig. 3*a*). The curve relating the size of the second response to the stimulus interval (Fig. 3*b*) shows a steep slope between 50 and 90 ms, with recovery to 50% of the

control value in about 60 ms. In contrast, Ca^{2+} transients measured with arsenazo III summate almost linearly with stimulus intervals as close as 20 ms (R. Miledi, I.P. and G. Schalow, unpublished data).

The abolition of the birefringence signals by intracellular injection of EGTA strongly suggests that the early signal is not associated with the Ca^{2+} release process by the SR, but is instead secondary to the increase in free sarcoplasmic Ca^{2+} concentration. This view is supported by the coincidence in time courses of the Ca^{2+} transient and the early birefringence signal, and by the experiment with the paired stimuli. The simplest interpretation of the paired stimulus experiment is that the early birefringence signal arises from a process which is fully saturated by the rise in sarcoplasmic Ca^{2+} concentration resulting from a single action potential. A possible mechanism for this early birefringence signal is a conformational change in the thin filament caused by the Ca^{2+} binding to troponin^{9,10}. Latency relaxation and elongation have recently been attributed to a lengthening of the thin filament resulting from Ca^{2+} binding, and the time course of these phenomena parallels that of the early optical changes^{1,3}.

This work was supported by grants from the MRC. G.S.K. was the recipient of a fellowship from CNPq, Brazil. We thank Professor R. Miledi for a critical reading of the manuscript.

G. SUAREZ-KURTZ*
I. PARKER

Department of Biophysics,
University College London,
Gower Street, London WC1, UK

Received 18 August; accepted 28 October 1977.

*Present address: Laboratory of Neurophysiology, College of Physicians and Surgeons, Columbia University, New York 10032.

- Hill, D. K. *J. Physiol., Lond.* **108**, 292-302 (1949).
- Eberstein, A. & Rosenfalck, A. *Acta physiol. scand.* **57**, 144-166 (1963).
- Barry, W. H. & Carnay, L. D. *Am. J. Physiol.* **217**, 1425-1430 (1969).
- Carnay, L. D. & Barry, W. H. *Science* **165**, 608-609 (1969).
- Bezanilla, F. & Horowicz, P. *J. Physiol., Lond.* **246**, 709-735 (1975).
- Baylor, S. M. & Oetliker, H. *Nature* **253**, 97-101 (1975).
- Oetliker, H., Baylor, S. M. & Chandler, W. K. *Nature* **257**, 693-696 (1975).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 141-162 (1977).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 163-198 (1977).
- Baylor, S. M. & Oetliker, H. *J. Physiol., Lond.* **264**, 199-213 (1977).
- Kovács, L. & Schneider, M. F. *Nature* **265**, 556-560 (1977).
- Miledi, R., Parker, I. & Schalow, G. *Proc. R. Soc. Lond. B* **198**, 201-210 (1977).
- Miledi, R., Parker, I. & Schalow, G. *J. Physiol., Lond.* **269**, 11-13P (1977).
- Ashley, C. C. *Am. Zool.* **7**, 647-659 (1967).

Calcium requirement for axoplasmic transport in mammalian nerve

To account for fast axoplasmic transport, the movement of materials in nerve fibres, a model has been advanced in analogy to the sliding filament mechanism of muscle contraction¹. We have shown in *in vitro* studies that transport is closely dependent on oxidative metabolism and a continual supply of ATP which could be hydrolysed by the Mg^{2+} - Ca^{2+} ATPase present in nerve². One might, therefore, expect a dependence of transport on either Ca^{2+} or Mg^{2+} . But, in previous studies of axoplasmic transport *in vitro*, we and others found transport to continue as usual when nerves were placed in incubation media free of divalent cations³⁻⁵. Some support for the involvement of Ca^{2+} in transport was provided by the fact that a block of transport was found with 50 mM oxalate, presumably by a binding of intracellular Ca^{2+} (ref. 1) and the block of organelle movement observed in single fibres exposed to 10 mM EDTA⁶. We report here that clear evidence for a participation of Ca^{2+} in axoplasmic transport was revealed when a desheathed nerve preparation was used.

The perineurial sheath acts as an effective permeability barrier in nerve^{7,8}, retaining ions including Ca^{2+} within the

endoneurial space. This could account for the apparent insensitivity of transport *in vitro* to a deficiency of Ca^{2+} in the medium when intact nerves are used. To examine the effect of Ca^{2+} on transport *in vitro*, it was essential that a long length of nerve be desheathed so as to allow the effects of a given concentration of Ca^{2+} or its absence from the medium to be assessed over incubation periods lasting at least 4 h. The desheathed nerve preparation used was the peroneal branch of the cat sciatic nerve from which a sufficiently long length of perineurial sheath can be readily removed.

In accord with our usual procedure, the L7 dorsal root ganglia were injected with $20 \mu\text{Ci}$ of ^3H -leucine which is rapidly taken up by the cell bodies and incorporated into proteins and polypeptides⁹. Two hours were allowed for proteins and polypeptides containing incorporated ^3H -leucine to move down the fibres of the tibial and peroneal nerve branches. The sciatic nerves were removed and the peroneal branches desheathed over a distance from 35 mm to 130 mm from the L7 dorsal root ganglia. The sciatic nerves with their desheathed peroneal and sheathed tibial branches attached were then put into flasks containing 20 ml of the desired solution and incubated for 3–5 h at 38°C while vigorously oxygenated with 95% O_2 + 5% CO_2 . The outflow patterns in both the peroneal and tibial branches were then separately determined by cutting each in equal 5 mm portions and assessing their content of labelled activity¹⁰.

With Ringer solution as the *in vitro* medium, or with isotonic NaCl or isotonic sucrose solution to which 5 mM Ca^{2+} was added, the outflow in both the desheathed peroneal and tibial nerves was similar (Fig. 1). The rate of transport

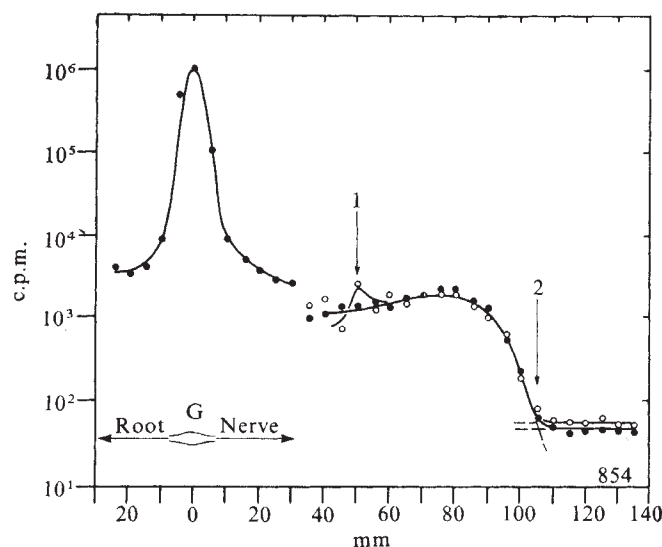


Fig. 1 Transport in medium containing 5 mM CaCl_2 . Outflow in the desheathed peroneal branch ($\circ$) and the sheathed tibial branch ($\bullet$) is shown. After 2 h of downflow *in vivo*, the peroneal nerve was desheathed and the nerve placed in an *in vitro* medium of 5 mM CaCl_2 and 140 mM NaCl for an additional 4 h. The points represent labelled materials present in 5 mm segments cut from the dorsal root, L7 ganglion (G) and nerve. Distal to 30 mm from the ganglion, each branch is cut individually and thus outflow in the tibial (T) and peroneal (P) branches can be shown separately. Arrow 1 indicates the upper end of the desheathed region and arrow 2, the front of the crests of transported material expected in both nerves. The crests seem to have the same height in this example. It should be noted that the logarithmic scale serves to diminish amplitude differences in the two branches. In general, a greater amount of labelled activity was usually found transported in the tibial branch since it is the larger of the two. A small degree of damming of activity is seen above the upper site where desheathing was initiated (arrow 1). Activity in counts per minute (c.p.m.) is given on the ordinate on a logarithmic scale. The position of sections is shown in mm from zero taken at the peak of activity in the ganglion.

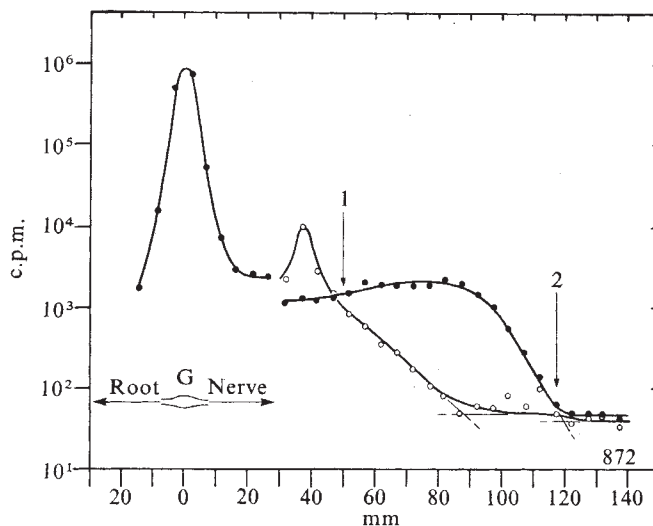


Fig. 2 Transport in Ca-free medium. Outflow in a sciatic nerve *in vitro* and its desheathed peroneal branch ($\circ$) and sheathed tibial branch ($\bullet$) is shown following injection of the L7 ganglion with ^3H -leucine. Downflow for 2 h was allowed in the animal before the sciatic nerve was removed, the peroneal branch desheathed and the nerve placed in an *in vitro* medium containing Ca-free isotonic NaCl. Above arrow 1, approximately where the peroneal nerve is desheathed, a peak of dammed activity is seen. This is followed by a steep descent to baseline at about 85 mm. Conversely, the tibial nerve shows a typical axoplasmic transport outflow. Arrow 2 marks the expected distance.

in both sheathed and desheathed branches determined in 25 experiments was close to 410 mm d^{-1} , the rate previously found to be characteristic of fast axoplasmic transport¹. A somewhat more sloping crest was seen at the advancing fronts in the desheathed nerves at lower Ca^{2+} concentrations of 1.5 to 3 mM. The addition of 4 mM K^+ to the medium with 1.5 Ca^{2+} gives a downflow closer to the usual pattern, indicating some participation of K^+ in maintaining transport at these Ca^{2+} concentrations.

With desheathed nerves placed in a Ca-free media, axoplasmic transport was blocked in the desheathed peroneal nerve (Fig. 2). The outflow of activity shows some damming at the upper end of the desheathed region followed by a gradual drop in the front of advancing activity until it meets the baseline level at which time a complete block of transport ensues. Subtracting the time of 2 h during which downflow took place in the animal, the block within the desheathed portion of the nerve was found to develop in 2.6 h of exposure to the Ca-free media (a, Table 1). A similar block was found when 4 mM EGTA was added to a Ca-free solution (b, Table 1) and to a Ringer solution in line with Ca^{2+} being the key factor. Little effect of variation of buffer and over pH 6.0–7.5 was found.

Transport was not maintained with Mg^{2+} in the incubation medium instead of Ca^{2+} (c, Table 1), indicating that Ca^{2+} is specifically required for the maintenance of axoplasmic transport. But, there was a small but significant prolongation of the time to block with Mg^{2+} present over the Ca-free medium which could indicate a partial effect of Mg^{2+} .

While axoplasmic transport was blocked in the desheathed nerve in the Ca-free medium, a normal appearing outflow was present in the sheathed tibial branch (Fig. 2). This is consistent with a large number of our previous observations showing an apparent lack of effect of Ca^{2+} depletion on axoplasmic transport in sheathed sciatic nerves *in vitro* and supports the view that Ca^{2+} is retained within the intact perineurial sheath.

We consider that the removal of Ca^{2+} from the medium lowers the level of free Ca^{2+} normally present in the fibres of desheathed nerves below that required for transport. The

level of free Ca^{2+} in the axoplasm of mammalian nerve fibres is not, however, known. If it is similar to that present in other cells, particularly the axoplasm of cephalopod giant axons, we would expect its concentration to be approximately 10^{-7} M (refs 11, 12). This low level of Ca^{2+} is considered to be maintained by several mechanisms in the face of a constant influx of Ca^{2+} . These include a Na^{+} - Ca^{2+} exchange carrier in the membrane¹³ which may possibly be dependent on ATP (refs 14, 15), a sequestration of Ca^{2+} within the mitochondrion^{16,17} and likely as well in the endoplasmic reticulum¹⁸. To this list we may add the possibility of a Ca-binding protein (CaBP) recently found in cat sciatic nerve¹⁹.

An involvement of mitochondria in the block of transport found in nerves placed in Ca-free media was indicated by a reduction in ATP and creatine phosphate levels. In nine nerves, the combined levels of ATP and creatine phosphate ($\sim\text{P}$) showed a decrease of approximately 22%, from control levels of $1.10 \pm 0.30 \mu\text{M}$ per g weight in a Ca-medium to $0.86 \pm 0.18 \mu\text{mol g}^{-1}$ in a Ca-free media which took place after 3 h. This degree of reduced $\sim\text{P}$ is not, however, likely in itself to account for the block of axoplasmic transport. In six desheathed nerves exposed to a Ca-free medium, we saw action potentials still remaining 2 h after a block of axoplasmic transport. This shows that the $\sim\text{P}$ remaining was sufficient to supply the Na-pump required to maintain excitability and presumably there should be an adequate supply for the transport mechanism as well. A block of axoplasmic transport and action potentials was found to occur only when $\sim\text{P}$ levels were reduced by 50% (ref. 20).

Table 1 Block time of axoplasmic transport on desheathed nerves in Ca^{2+} -free media

	Condition	n	Block time (h)
a	Ca^{2+} -free	24	2.6 ± 0.79
b	EGTA + Ca^{2+} -free	11	3.0 ± 0.36
c	5 mM Mg^{2+}	15	3.5 ± 0.48

The time to block is expressed in hours calculated from the following equation: $(D_2 - D_1) \text{ mm} / 17.4 \text{ mm h}^{-1}$, where D_2 = total distance the labelled materials moved down in the sheathed and desheathed part of the peroneal branch; D_1 = distance moved in the sheathed part of the peroneal branch. All media containing NaCl or sucrose so that the isotonicity was adjusted to approximately 300 mOsm. 10 mM NaHCO_3 was added as buffer (pH 6.7). n, No. of experiments; P = significance. The significant levels were analysed by *t*-test. P values for groups a, c < 0.001, for b, c < 0.01, and for a, b > 0.2. Values are mean $\pm$ s.d.

Transport in the desheathed nerve also showed a progressive failure of transport with higher than normal levels of Ca^{2+} present in the incubation medium. This was seen to occur in the range of 25–100 mM Ca^{2+} . At the highest levels of Ca^{2+} (95–100 mM) transport was blocked in a little less than 2 h of incubation. The actual amount of Ca^{2+} entering the fibres and the resulting concentration change in the axoplasm is unknown. These findings suggest, however, that Ca^{2+} must be regulated within some limited range for normal transport.

An excess of Ca^{2+} entering the fibres could cause a block of transport by interfering with ATP production^{16,17} or its utilisation by the Mg^{2+} - Ca^{2+} ATPase. In addition, at the higher levels of Ca^{2+} , preliminary electron microscopic studies have shown a marked depletion of microtubules in the fibres²⁰, which may be related to the disassembly of isolated microtubules by Ca^{2+} (S.O., S.-Y.C., R. Jersild and V. McAdoo, unpublished and ref. 21). On the basis of the transport filament hypothesis a block of ATP production or its utilisation, and at higher Ca^{2+} levels the disassembly of microtubules, would all be possible causes for the block of axoplasmic transport observed.

We thank Debbie Jadhav and Larry Smith for their help and the Illustrations Department for the figures. This work was supported by NIH PHS RO1 NS 8706-07 and NSF BNS 75-0368-A03.

SIDNEY OCHS
ROBERT M. WORTH
SHEW-YIN CHAN

Department of Physiology and the
Medical Biophysics Program,
Indiana University School of Medicine,
Indianapolis, Indiana

Received 19 August; accepted 24 October 1977.

- Ochs, S. *Science* **176**, 252–260 (1972).
- Khan, M. A. & Ochs, S. *Brain Res.* **81**, 413–426 (1974).
- Banks, P., Mayor, D. & Mraz, P. *J. Physiol., Lond.* **229**, 383–394 (1973).
- Ochs, S. & Smith, C. *J. Neurobiol.* **6**, 85–102 (1975).
- Hammerschlag, R., Dravid, A. R. & Chiu, A. Y. *Science* **188**, 273–275 (1975).
- Kirkpatrick, J. B. & Rose, R. E. *Trans. Soc. Neurosci.* **2**, 255 (1972).
- Crescitelli, F. *Ann. J. Physiol.* **166**, 229–240 (1951).
- Krujević, K. *J. Physiol., Lond.* **128**, 473–488 (1955).
- Ochs, S., Sabri, M. I. & Johnson, J. *Science* **163**, 686–687 (1969).
- Ochs, S. *J. Physiol., Lond.* **227**, 627–645 (1972).
- Baker, P. F. *Prog. Biophys. molec. Biol.* **24**, 177–223 (1972).
- Blaustein, M. P. *Rev. Physiol. Biochem. Pharm.* **70**, 33–82 (1974).
- Brinley, F. Jr, Spangler, S. G. & Mullins, L. J. *J. gen. Physiol.* **66**, 223–250 (1975).
- Baker, P. F. *Fedn Proc.* **35**, 2589–2595 (1976).
- DiPolo, R. *Fedn Proc.* **35**, 2579–2582 (1976).
- Lehninger, A. L. *Biochemistry*, 2nd edn (Worth, New York, 1975).
- Carafoli, E. & Crompton, M. *Symp. Soc. exp. Biol.* **30**, 89–115 (1976).
- Stöckel, M. E., Hindelang-Gertner, C., Dellmann, H. D., Porte, A. & Stutinsky, F. *Cell Tiss. Res.* **157**, 307–322 (1975).
- Iqbal, Z. & Ochs, S. *Soc. Neurosci. Abst.* **2**, 47 (1976).
- Ochs, S. *Fedn Proc.* **33**, 1049–1058 (1974).
- Weisenberg, R. C. *Science* **177**, 1104–1105 (1972).
- Shelanski, M. L., Gaskin, F. & Cantor, C. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 765–768 (1973).

Ionic radius selectivity of skeletal muscle membranes

THERE have been several reports^{1–3} that the trivalent lanthanide cations bind to sites on membranes that are normally occupied by Ca^{2+} . In skeletal muscle, Ca^{2+} is important for the functions of both the outer sarcolemma⁴ and the internal sarcoplasmic reticulum^{5,6}. Here I report the inhibitory effects of 13 lanthanide ions on these Ca^{2+} -mediated functions. The relationship between ionic radius and the degree of inhibition is different in these two membranes. There are features of the data which are difficult to explain by assuming that charge density alone determines cation recognition at the membrane surface. But it would also be too simplistic to suggest that the lanthanide ions are selected only on the basis of ionic radius.

One of the main functions of the sarcolemma is the propagation of the action potential over the surface of the muscle fibre, causing it to contract. It has been reported that the maintenance of the action potential depends on membrane-bound Ca^{2+} and that this Ca^{2+} is specifically displaced by lanthanum ions⁴. Lanthanide ions have been shown⁷ to exert a strong inhibition on the twitch response of small bundles (3–5 fibres) of toad semitendinosus fibres. The site of action of these ions seems to be the sarcolemma because they have been localised there and because removal of the sarcolemma by glycerination substantially raises the threshold concentration needed to inhibit contraction⁷.

Inhibition of the twitch response is most conveniently expressed as the concentration of lanthanide ion required to produce 50% inhibition of the twitch tension. Figure 1 shows that most of the lanthanide ions have about the same effect on twitch tension except for three, Tm^{3+} , Er^{3+} and to a lesser extent Ho^{3+} , which are required in significantly higher concentrations in order to effect a 50% reduction in the contractile response. These ions have ionic radii of 99, 100 and 102 pm respectively and therefore correspond exactly with the published⁸ radius of Ca^{2+} (100 pm). These data could be interpreted to mean that the skeletal muscle sarcolemma possesses an extremely acute ability to detect,

within a few pm, the ionic radii of multivalent cations such as the trivalent lanthanide ions.

The acute sensitivity of the sarcolemma to ionic radii of the lanthanide ions raises the question of whether this property is shared with other muscle membranes. Skeletal muscle sarcoplasmic reticulum is primarily concerned with the release of Ca^{2+} during activation of the fibre and with the subsequent recovery of Ca^{2+} associated with relaxation. *In vivo* it exists as a complex of flat fenestrated sheets and sacs surrounding the myofibrils. It can, however, be isolated in the form of vesicles which have been well characterised⁵. The membranes of these vesicles are structurally specialised and can transport Ca^{2+} against concentration gradients of up to 10,000 by the hydrolysis of Mg-ATP (ref. 6). Recent results³ have shown that (1) lanthanide ions such as Gd^{3+} cannot penetrate the vesicles provided that their membranes are not damaged; (2) Gd^{3+} inhibits the Ca^{2+} -stimulated Mg^{2+} -dependent ATPase activity associated with Ca^{2+}

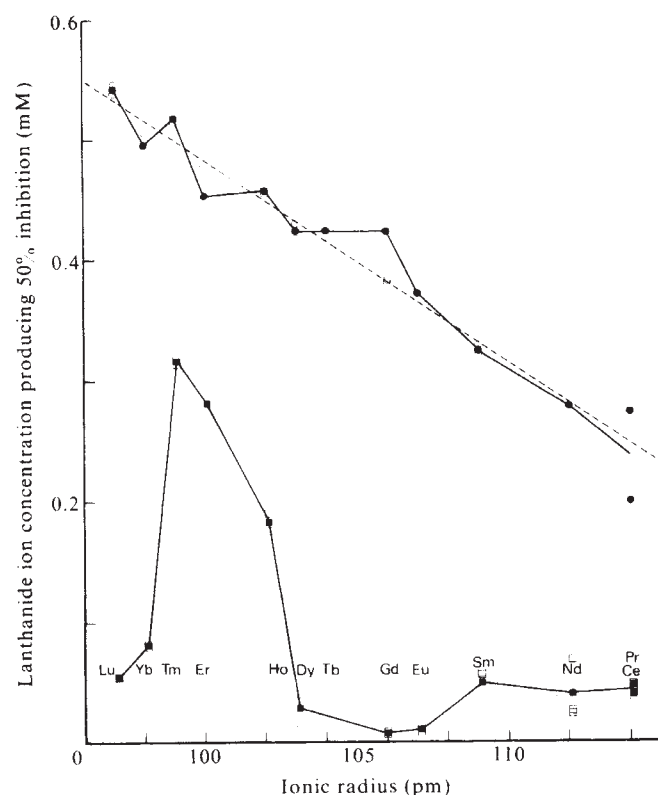


Fig. 1 Relationship between ionic radius of the lanthanide ions (assuming a coordination number of 8 (ref. 11)) and the functions of two different membrane systems from vertebrate skeletal muscle. The upper curve represents the inhibition of Ca^{2+} -stimulated Mg-ATPase activity of isolated rabbit sarcoplasmic reticulum vesicles, expressed as the concentration of added lanthanide ion required to inhibit this activity by 50%. This concentration dependence (0.5×10^{-4} – 5.0×10^{-4} M lanthanide ion) was determined at least twice for each of the 13 lanthanide ions ($\circ$) and the solid line joins their means ($\bullet$). The dotted line is the linear regression ($r=0.972$) fitted to the means. Conditions for these experiments were: KCl, 0.1 M; HEPES, 0.01 M; ATP, 2 mM; MgCl_2 , 2.5 mM; sarcoplasmic reticulum protein, 0.1 mg ml⁻¹; pH 7.0 at 25 °C (see ref. 3 for further details). The lower curve relates the ionic radii of the 12 lanthanide ions tested to the added concentration of each ion required to inhibit twitch tension by 50%. This concentration dependence (0.2×10^{-4} – 10×10^{-4} M lanthanide ion) was determined at least twice for each of the ions ($\square$) and their means ($\blacksquare$) have been joined by the solid line. Experimental conditions were: 3–5 toad semitendinosus fibres supramaximally stimulated in NaCl, 115 mM; KCl, 2.5 mM; CaCl_2 , 1.8 mM; HEPES, 5 mM; pH 7.0 at 25 °C (for further details see ref. 7). The binding of the lanthanide ions to either the sarcolemma or the sarcoplasmic reticulum was found to be completely reversible.

uptake; and (3) the concentration dependence of this inhibition is less sharp than that observed in the sarcolemma experiments⁷. Therefore a series of experiments analogous to those described with the sarcolemma was undertaken. The data (Fig. 1) are a contrast to the sarcolemma experiments. There is no abrupt effect near the radius of Ca^{2+} . Instead, there is a roughly linear relationship between ionic radius and inhibition of sarcoplasmic reticulum Mg-ATPase activity.

Now that there are data on the ionic radius dependence of lanthanide ion inhibition of the sarcoplasmic reticulum, they can be compared with those from the sarcolemma experiments⁷. Clearly the two membranes behave quite differently in the presence of the lanthanide ions. The sarcolemma exhibits a sharp break at the ionic radius of Ca^{2+} which is absent from the sarcoplasmic reticulum data. The ionic milieu inside the muscle fibre is essentially constant, having high concentrations of Mg^{2+} and K^{+} and low Ca^{2+} and Na^{+} . Only during activation is it necessary for the outer surface of the sarcoplasmic reticulum membrane to distinguish between Ca^{2+} (100 pm) and Mg^{2+} (49 pm). The outer surface of the sarcolemma is, however, confronted with high concentrations of Mg^{2+} , Ca^{2+} and Na^{+} and low K^{+} , and there may be other cations present in the fluid around the muscle cells which will complicate the need for ionic selectivity. Perhaps it is not surprising that the sarcolemma has a more acute ability to recognise cations the size of Ca^{2+} and that the sarcoplasmic reticulum exhibits only a broad selectivity with respect to ionic radius.

What information do these data yield about the mechanism by which sites on muscle membranes recognise cations like Ca^{2+} ? The lanthanide ions in some respects resemble Ca^{2+} , for not only do their ionic radii overlap, but both exhibit purely ionic interactions under biological conditions¹¹. It is tempting to conclude that these membranes select multivalent cations on the basis of ionic radius as the data (particularly the sarcolemma experiments) suggest. But all the lanthanide ions inhibit the Ca^{2+} -mediated functions in these membranes and so it seems that these membranes can distinguish between Ca^{2+} and those lanthanide ions with nearly similar ionic radii. Moreover, although the term "ionic radius" is valuable as a generalised property of ions, it is perhaps misleading if interpreted too precisely, for in reality there is no radius, but rather a set of dimensions which can vary depending on the ligand^{8,9}. On the other hand, charge density¹⁰ also seems unlikely to be the criterion for selecting ions like Ca^{2+} , since the larger lanthanide ions have approximately the same charge density as Ca^{2+} but are not apparently distinctive in their effects on the sarcolemma membrane. If neither ionic radius nor charge density alone can account for these data then perhaps some other explanation should be sought.

This research was supported by grants from the National Heart Foundation of Australia and from the National Health and Medical Research Council. I thank Mr Brett Hambly and Miss Janice Lange for their assistance.

C. G. DOS REMEDIOS

Muscle Research Unit,
Department of Anatomy,
The University of Sydney,
Sydney, NSW 2006, Australia

Received September; accepted 28 October 1977.

- Mela, L. *Biochemistry* 8, 2481–2486 (1969).
- Lehninger, A. L. & Carafoli, E. *Archs Biochem. Biophys.* 143, 506–515 (1971).
- dos Remedios, C. G. *J. Biochem.* 81, 703–709 (1977).
- Andersson, K. E. & Edman, K. A. P. *Acta physiol. scand.* 90, 113–123 (1974).
- Inesi, G. A. *Rev. Biophys. Bioengng* 1, 191–210 (1972).
- Hasselbach, W. *Biophys. Struct. Mech.* 3, 43–54 (1977).
- Hambly, B. D. & dos Remedios, C. G. *Experientia* 33, 1012–1014 (1977).
- Shannon, R. D. & Prewitt, C. T. *Acta Crystallogr.* B25, 925–946 (1969).
- Williams, R. J. P. *Symp. Soc. exp. Biol.* 30, 1–17 (1976).
- Diamond, J. M. & Wright, E. M. A. *Rev. Physiol.* 31, 581–646 (1969).
- Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P. & Xavier, A. V. *Nature* 232, 236–245 (1971).

Control of interaction of spectrin and actin by phosphorylation

SPECTRIN is a high molecular weight protein located on the cytoplasmic surface of the mammalian erythrocyte membrane, from which it may be readily liberated by extraction with solutions of low ionic strength. It is thought to be a major structural element of the cell and to play a critical part in maintaining its discoid shape and characteristic viscoelastic properties. Birchmeier and Singer¹ have shown that changes in the shape of red cell ghosts are associated with the phosphorylation of a single serine residue on one of the two spectrin subunits, and have suggested that this might provide a basis for the well-known control of red cell shape by ATP². The erythrocyte membrane also contains actin, which is present in approximately equimolar proportions to the spectrin dimer (molecular weight 500,000 (ref. 3)). We have shown previously¹ that there is a specific interaction between these two proteins, which reveals itself in the ability of spectrin to provoke the polymerisation of muscle actin *in vitro*. We show here that this effect depends on the phosphorylation of spectrin. Furthermore, in an undispersed mixture of spectrin and its cognate actin such phosphorylation causes the formation of a gel. The striking parallel between these results and the previously demonstrated effects of phosphorylation *in situ* suggests that the shape of the cell is controlled primarily by the actin-spectrin complex.

At least two kinase activities have been recognised in human erythrocyte membranes^{3,6}. Since the phosphorylation of spectrin in the intact cell does not require cyclic AMP, we isolated the cyclic AMP-independent kinase, following the method of Hosey and Tao⁷, and tested its ability to phosphorylate spectrin in solution. Ghosts were prepared from human erythrocytes, not more than 1-d-old, by the procedure of Hanahan *et al.*⁸. Spectrin was extracted from water-washed ghosts either by agitation for 1 h at 37 °C or by a 24-h dialysis in the cold, and purified by gel filtration⁹. In the presence of γ -labelled ³²P-ATP and kinase, about 0.45 g atom of phosphorus per mol of spectrin was incorporated (Fig. 1a). Autoradiography of this material after separation on sodium dodecyl sulphate (SDS)-acrylamide gels confirms that the phosphate is located exclusively on the small subunit (Fig. 1b) as in endogenously phosphorylated spectrin^{1,3}.

The effect of this phosphorylation on the spectrin-induced polymerisation of actin is dramatic (Fig. 2). A very rapid rise in viscosity is observed in conditions in which actin itself remains monomeric. The increase occurs without a detectable lag phase, and the reduced viscosity reaches a final level of 0.9–1.2 ml mg⁻¹ at an actin concentration of 0.5–0.6 mg ml⁻¹, which is close to that achieved in the salt-induced polymerisation of actin alone. Phosphorylated spectrin containing about 0.45 g atom phosphorus per mol of protein produced a partial rise time 30 to 100 times that given by normally prepared spectrin. It was shown previously that the increase in viscosity is accompanied by the appearance of bundles of thin filaments composed principally of actin⁴, as well as some spectrin. We have now found that it is the phosphorylated form that is preferentially associated with the polymerised actin: thus in two experiments, the specific activity of ³²P-labelled spectrin carried down with the actin on centrifugation was more than 50 times that remaining in the supernatant.

Treatment of the spectrin with bacterial alkaline phosphatase causes a loss of the phosphate group and almost entirely eliminates the capacity to induce actin polymerisation. That this is not a consequence of damage by endogenous proteolytic activity is shown by its reversibility: if kinase is added after the phosphatase, phosphorylation

occurs and polymerisation of actin ensues as before (Fig. 2). Relatively high concentrations of phosphatase are used in these experiments, spectrin being apparently a poor substrate for this bacterial enzyme. In the absence of phosphatase, storage of purified spectrin in buffer caused no

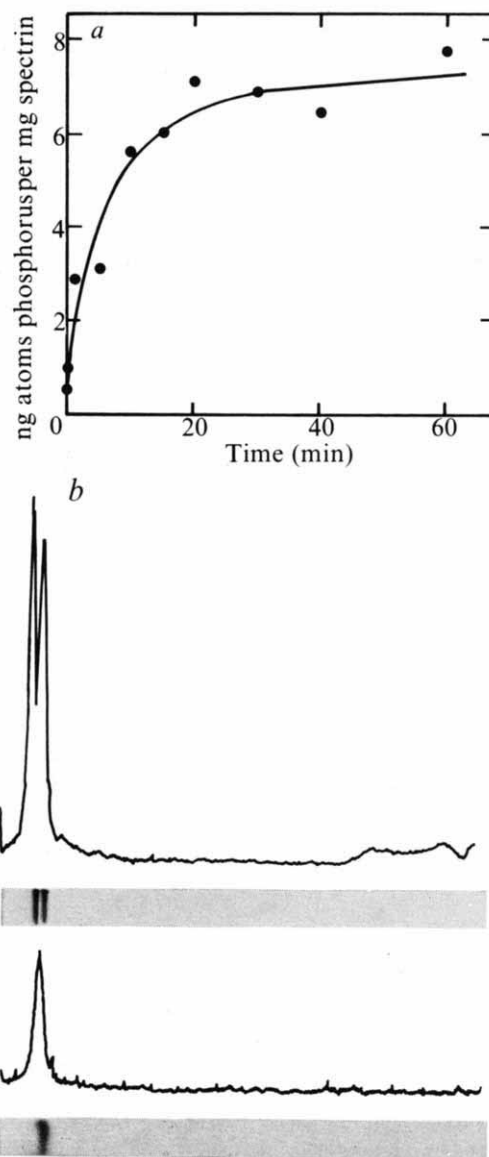


Fig. 1 *a*, Phosphorylation of spectrin in solution by red cell membrane kinase. Crude spectrin extract was dephosphorylated with bacterial alkaline phosphatase (compare Fig. 2), and purified by column chromatography on Sephadex G200⁹; 100 μ l crude human erythrocyte membrane kinase⁷ in 20mM Tris, 1mM dithiothreitol, pH 7.4, at 3 mg ml⁻¹ total protein concentration (estimated by colorimetric micro-Kjeldahl analysis¹⁰), was added to 0.6 mg dephosphorylated spectrin in 100mM sodium chloride, 10mM Tris, 5mM magnesium chloride, pH 7.4 at 4 °C. Phosphorylation was started by the addition of 1 μ l γ -³²P-ATP of specific activity 1 mCi ml⁻¹ and the mixture incubated at 37 °C; 50- μ l aliquots were removed at intervals, spotted on to squares of Whatman No. 1 filter paper and air-dried. Labelled protein was fixed and excess radioactivity removed by washing in two changes of cold 5% trichloroacetic acid (TCA) for 15 min each time, followed by boiling for 15 min and a further cold TCA wash. The paper squares were oven-dried at 60 °C and samples solubilised for counting by adding 0.5 ml Soluene (Packard) and incubating overnight at 20 °C. Then 3 ml scintillation fluid containing 5.5 g Permablend (Packard) and 1 ml acetic acid per l toluene were added and the samples counted in a Nuclear Chicago liquid scintillation counter. The data are corrected for background counts. *b*, Autoradiograph of gel electrophoresis in SDS of membrane protein extract after phosphorylation (lower), and stained gel (upper), with densitometer traces. This shows that only spectrin has been sensibly phosphorylated, and that the ³²P is incorporated exclusively in the smaller subunit.

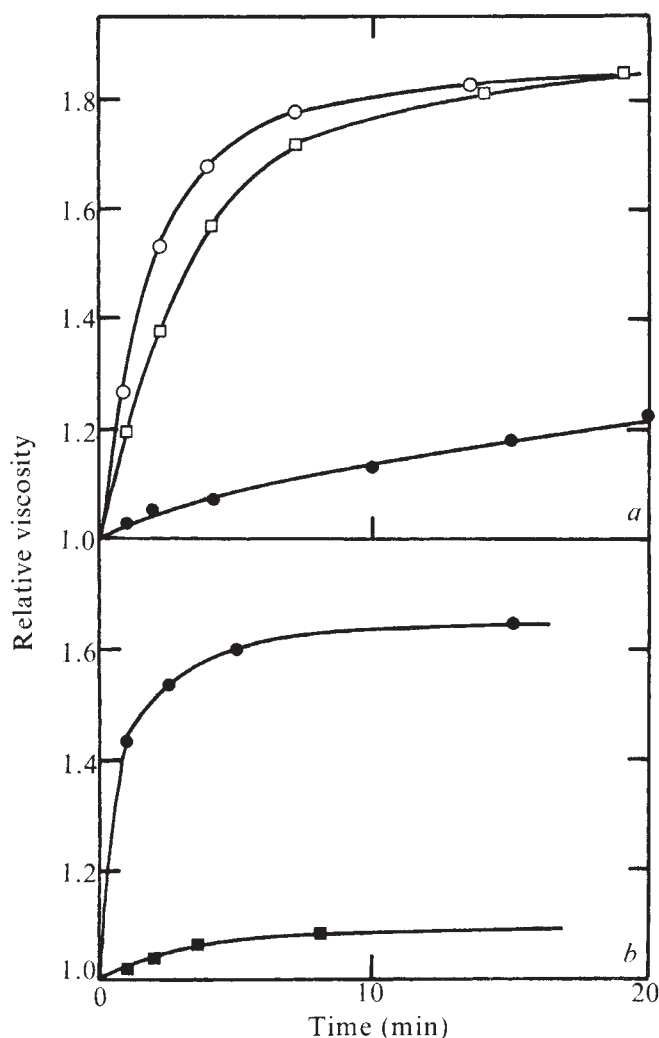


Fig. 2 Effect of phosphorylation state of spectrin on polymerisation in actin-spectrin mixtures. Actin concentration, 0.6 mg ml^{-1} throughout. *a*, Effect of phosphorylation with red cell membrane kinase on time course of polymerisation: spectrin as prepared untreated (●), after treatment with kinase (○), and after serial exposure to alkaline phosphatase, and kinase (□). The spectrin concentration in (*a*) is 0.04 mg ml^{-1} . *b*, Effect of dephosphorylation with alkaline phosphatase on time course of polymerisation: spectrin as prepared, untreated (●), and after treatment with phosphatase (■). Spectrin concentration in (*b*), 0.4 mg ml^{-1} . The spectrin was prepared by extraction at 37°C for 1 h¹⁷, and purified by column chromatography on Sephadex G200[®]. The G-actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt¹⁸. The procedure for viscometry and the solvent conditions were as described previously⁴—Ostwald capillary viscometer with water flow time of 40 s, maintained at 30°C ; solvent was 10 mM sodium phosphate, 0.1 mM calcium chloride, 0.2 mM ATP, 0.2 mM dithiothreitol, pH 8.2. Kinase-catalysed phosphorylation of spectrin was allowed to proceed for 30 min in the presence of 1 mM ATP (see Fig. 1). Spectrin was dephosphorylated by the addition of 0.25 mg bacterial alkaline phosphatase (Worthington) per mg spectrin in 10 mM Tris, 140 mM potassium chloride, 20 mM sodium chloride, pH 7.4, followed by incubation at 37°C for 2 h. After treatment with the enzymes the spectrin was dialysed in the cold into the buffer used for viscometry. Note the 10-fold difference in spectrin concentration between (*a*) and (*b*) to allow comparison of untreated with both kinase and phosphatase-treated material.

perceptible loss of phosphorus in periods of up to some days. Treatment of actin alone, with either enzyme, had no effect on its polymerisation properties.

In our previous study⁴ we found that the ability to cause actin polymerisation was shown only by spectrin prepared from fresh red blood cells, and even then the response showed some variability. By contrast, the spectrin phosphorylated as described above induced polymerisation in a

consistent and reproducible fashion. Indeed, inactive preparations of spectrin, such as those prepared from stored blood, have been reactivated by phosphorylation. It seems, therefore, that the previously observed variability was due to a failure to control the phosphorylation state of spectrin.

We have attempted to establish whether the phosphorylation-dependent interaction between spectrin and muscle actin has a counterpart in the erythrocyte. It is known that the bulk of the membrane protein and lipid components can



Fig. 3 Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulphate (SDS), of cytoskeletal extract from human erythrocyte membranes. Washed ghosts were prepared by the method of Dodge *et al.*⁸, but with inclusion in the lysis buffer of 0.2 mM dithiothreitol, 0.2 mM ATP calcium chloride; 5 ml of the undiluted washed ghost pellet were added to 20 ml 1% Triton X-100, containing 25 mM Tris, 0.2 mM ATP, 0.2 mM calcium chloride, pH 7.4, mixed gently and incubated at 37°C for 30 min to maximise extraction of lipid. The pellet obtained after centrifugation at $80,000g$ for 3 h was solubilised by heating at 100°C for 5 min in 0.125 M Tris, 1% SDS, pH 6.8, and subjected to electrophoresis in an 8% polyacrylamide gel using a discontinuous Tris-glycine buffer system containing 0.1% SDS¹⁹. The gels were stained with 0.05% Coomassie brilliant blue R in methanol-acetic acid-water (5:1:5 v/v), and de-stained in 10% acetic acid.

be extracted from human erythrocyte ghosts with the non-ionic detergent Triton X-100 (ref. 10). Such extraction leads to what in the electron microscope appears as a fibrous network, retaining the outline of the shape of the ghosts¹⁰. When this residue is examined by SDS-acrylamide gel electrophoresis, the major components are found to be actin and spectrin (Fig. 3). The Triton-extracted ghost residues may be dispersed by gentle homogenisation in buffer to give a mobile suspension with a total protein concentration of 20 mg ml^{-1} . When the kinase preparation is added to this suspension at 37°C , a stiff gel is rapidly formed (Fig. 4). This phenomenon not only depends on

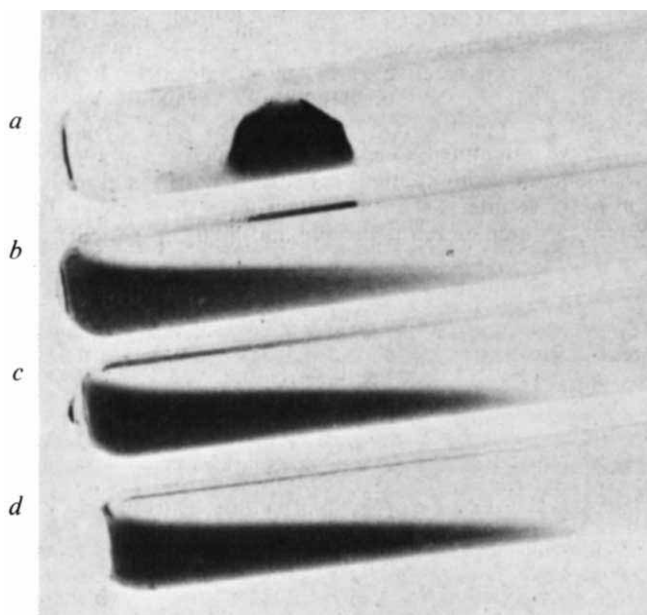


Fig. 4 Phosphorylation-dependent gelation of cytoskeletal spectrin-actin residue. Pellets prepared from Triton X-100 extract of ghosts (see Fig. 3) containing 20 mg total protein (equivalent to 10 ml ghost suspension), as estimated by Folin-Ciocalteu analysis, were homogenised gently in 10 mM Tris, 150 mM potassium chloride, 5 mM magnesium chloride, 1 mM ATP, pH 7.4, to give a total volume of 1 ml. The tubes contain this mixture; *a*, after addition of 3 mg crude red cell membrane kinase; *b*, untreated; *c*, after addition of 1 mg DNAase I (Sigma), followed by incubation at 37°C for 30 min, and addition of 3 mg kinase; *d*, as (*c*) but in the absence of kinase. All mixtures were then maintained at 37°C for 30 min. The gelation observed in (*a*) occurred within 5 min.

phosphorylation by the kinase, but also requires the presence of the actin: this is demonstrated by the introduction of the specific actin-sequestering protein, deoxyribonuclease I^{11,12}. If this is added to a concentration about equimolar to that of actin, phosphorylation then fails to induce any formation of gel, or any other macroscopically observable effect (Fig. 4).

The formation of the gel by the cytoskeletal extract suggests that phosphorylation leads to a cross-linked complex of spectrin and actin; the inhibitory effect of deoxyribonuclease indicates that actin is directly implicated in the change of state, but does not prove that this involves a polymerisation, rather than an association between spectrin and actin monomers. It may be relevant in this context to note that muscle and erythrocyte actin are not identical, and may be distinguished by two-dimensional separation on a polyacrylamide gel¹³.

The results described here extend our earlier inference of a specific functional relationship between spectrin and actin in the erythrocyte membrane⁴. Although spectrin has so far been identified only in the red blood cell¹⁴, gelation phenomena have been observed in systems from several other kinds of cells, involving actin and high molecular weight proteins (see ref. 15 for review). These are of great interest because they could reflect mechanisms by which actin polymerisation is controlled in the cell. The requirement for ATP by these gelation reactions may be an indication that, as in the system described here, phosphorylation of one of the protein components is mandatory.

We thank Mr C. Woodfine and the staff of St Paul's Hospital for supplying the blood used in this work.

JENNIFER C. PINDER
D. BRAY
W. B. GRATZER

MRC Cell Biophysics Unit,
King's College,
Drury Lane,
London WC2, UK

Received 9 September; accepted 14 November 1977.

1. Birchmeier, W. & Singer, S. J. *J. Cell Biol.* **73**, 647-659 (1977).
2. Makato, N., Nakao, T. & Yamazoe, S. *Nature* **187**, 945-946 (1960).
3. Gratzer, W. B. & Beaven, G. H. *Eur. J. Biochem.* **58**, 403-409 (1975).
4. Pinder, J. C., Bray, D. & Gratzer, W. B. *Nature* **258**, 765-766 (1975).
5. Rubin, C. S., Erlichman, J. & Rosen, O. M. *J. Biol. Chem.* **247**, 6135-6139 (1972).
6. Avruch, J. & Fairbanks, G. *Biochemistry* **13**, 5507-5514 (1974).
7. Hosey, M. M. & Tao, M. *Biochim. biophys. Acta* **482**, 348-357 (1977).
8. Dodge, J. T., Mitchell, C. & Hanahan, D. J. *Archs Biochem.* **100**, 119-130 (1963).
9. Pinder, J. C., Tidmarsh, S. & Gratzer, W. B. *Archs Biochem.* **172**, 654-660 (1976).
10. Yu, J., Fischman, D. A. & Steck, T. L. *J. supramol. Struct.* **1**, 233-248 (1973).
11. Lazarides, E. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4742-4746 (1974).
12. Hitchcock, S. E., Carlsson, L. & Lindberg, U. *Cell* **7**, 531-542 (1976).
13. Garrels, J. I. & Gibson, W. *Cell* **9**, 793-805 (1976).
14. Hiller, G. & Weber, K. *Nature* **266**, 181-183 (1977).
15. Hitchcock, S. E. *J. Cell Biol.* **74**, 1-15 (1977).
16. Jaenicke, L. *Analyt. Biochem.* **61**, 623-627 (1974).
17. Marchesi, V. T. *Meth. Enzym.* **32**, 275-277 (1974).
18. Spudich, J. A. & Watt, S. J. *J. Biol. Chem.* **246**, 4866-4871 (1971).
19. Laemmli, U. K. *Nature* **227**, 680-685 (1970).

Formation of branched DNA structures by *Xenopus laevis* oocyte extract

We have reported that a soluble cell-free extract from stage 6 oocytes of *Xenopus laevis* can act on supercoiled SV40 DNA to produce molecules with a single-strand scission, linear molecules of full length, shorter fragments and various forms of complex DNA¹. The complex DNA consisted of various structures, such as figure-of-eight dimers, catenated dimers, circular dimers, catenated trimers, late Cairns' structures, complex multimers and circular monomers with tails. The few late Cairns' structures observed could have been due to replication, but it is possible that they and the other forms of complex DNA resulted from recombination among SV40 DNA molecules. We thought that fractionation of the extract should produce fractions lacking some of the activities necessary to produce complex DNA, so that intermediate structures would accumulate. We now report that some fractions of the extract produce a high percentage of branched DNA structures from SV40 DNA, and we describe the requirements and characteristics of this reaction. We also discuss the possibility that branched DNA is an intermediate in the formation of complex DNA.

The extract was fractionated and prepared as described in the legend to Fig. 1. The fractions eluting from the phosphocellulose column at 0.2-0.25 M KCl produced nicked circles, linear molecules and branched DNA. The branched DNA was very varied in form (circular and linear), length, number and arrangement of branches (Fig. 1). The length of the molecules in the branched structures, in view of the length of the SV40 DNA, indicated that more than one molecule was involved.

Table 1 Effect of temperature and time of incubation on the formation of branched structures

Temperature (°C)	Time (min)	% Branched structures	No. molecules scored
30	60	3.6	1,110
	120	11.8	1,180
37	60	8.9	1,120

Supercoiled SV40 DNA at 14 µg ml⁻¹ was incubated with the phosphocellulose fraction in the conditions indicated in Fig. 1.

There were often several branches in one structure; because of the high dilution used for the electron microscopy preparation, these multiple-branched forms cannot be due to random association. The structures formed without added deoxytriphosphates, and it was highly unlikely that any endogenous deoxytriphosphates remained in the conditions used. Thus branched DNA structures were probably formed in the absence of DNA synthesis.

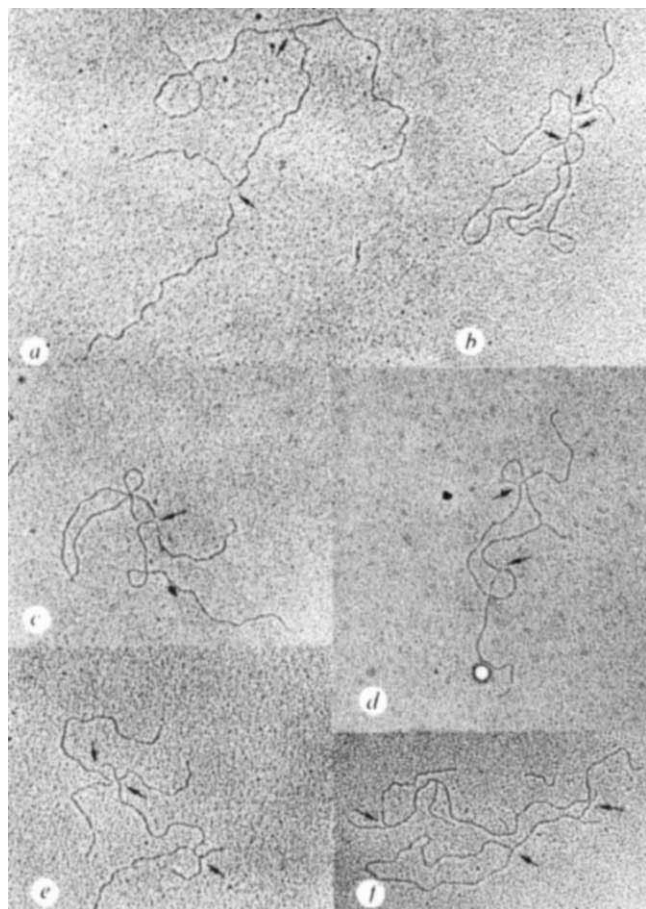


Fig. 1 Electron micrographs of branched structures produced by incubating supercoiled SV40 DNA with a phosphocellulose fraction. Supercoiled SV40 DNA was prepared as previously described¹. To prepare the phosphocellulose fractions, 20 ml of collagenase-treated stage 6 oocytes (about 14,000 cells) were used. All operations were carried out at 0–4 °C. The oocytes were homogenised in a Dounce homogeniser with 80 ml of TEMG buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1.4 mercaptoethanol, 20% (w/v) glycerol). The homogenate was centrifuged for 15 min at 8,000g and the supernatant, after removal of the floating lipid layer, was centrifuged in a Beckman 50 Ti rotor at 40,000 r.p.m. for 60 min. The high-speed supernatant was brought to 60% saturation with ammonium sulphate in the cold. The precipitate was collected by centrifugation, dissolved in 15–20 ml of TEMG buffer, and dialysed exhaustively against TEMG. The dialysate was centrifuged for 30 min at 30,000g to remove insoluble proteins and applied to a DEAE-cellulose column (Whatman DE-52) (3 × 20 cm) equilibrated with TEMG buffer. The column was washed with 3 volumes of buffer and eluted with TEMG containing 0.2 M KCl. All the fractions containing proteins, as detected by absorbance at 280 nm, were pooled (50–60 ml) and applied to a phosphocellulose column (1 × 5 cm) equilibrated with TEMG buffer. The column was washed with 3 volumes of buffer and subsequently eluted with 40 ml of a linear gradient of 0–1.0 M KCl in TEMG containing bovine serum albumin (0.5 mg ml⁻¹). Fractions (1.5 ml) were collected, dialysed against TEMG buffer, divided in aliquots and stored at –70 °C. The dialysed fractions were assayed for the formation of branched structures in a reaction mixture containing in a volume of 50 µl, 50 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 6 mM dithiothreitol, 0.1–0.5 µg of SV40 DNA and 25 µl of the phosphocellulose gradient fractions. After incubation for 60 min at 30 °C, the reaction was stopped by addition of sodium dodecyl sulphate and EDTA to final concentrations of 1% and 15 mM respectively. Distilled water (50 µl) was added to each reaction mixture and the DNA was extracted with phenol and ether and used directly for electron microscopy or after concentration by ethanol precipitation. Samples were prepared as previously described¹ (× 6,100). Arrows indicate branch points.

The formation of branched structures was absolutely dependent on the presence of magnesium. In the absence of ATP, we observed extensive degradation of the DNA. We therefore conclude that degradation is inhibited in the presence of ATP, but we could not show that ATP was involved in the formation of branched structures. Addition of the four deoxytriphosphates did not increase the proportion of branched structures, indicating further that DNA synthesis is not required.

Table 1 shows the effect of temperature and time of incubation on the formation of branched structures. The percentage of branched structures after 60 min of incubation at 37 °C was at least twice the percentage of branched structures formed at 30 °C. The reaction was time dependent: four times as many branched structures formed after 120 min at 30 °C as after 60 min. The generation of multiply-branched structures showed the same time dependence as did that of molecules with a single branch.

Because more than one molecule was involved in each branched structure the percentage of these forms should have increased with increasing DNA concentration. This was the case with concentrations up to 3 µg ml⁻¹. At higher DNA concentrations the percentage of branched structures decreased, suggesting that, because the reaction was presumably catalysed by many factors, some of these became limiting at increasing DNA concentrations.

Supercoiled DNA was not required for the formation of branched structures (Table 2). We have observed that relaxed molecules, prepared by the action of *Xenopus laevis* DNA-relaxing enzyme¹, are four times more efficient than the supercoiled form; both nicked circles with a single-strand scission and linear molecules react with approximately the same efficiency. We cannot explain the relatively low efficiency of supercoiled DNA. Table 2 shows a correlation among multiple branches in a single structure; when linear molecules were used as substrate, 20.5% of the 500 structures observed were

branched. An independent origin of each branch would have led to $(0.205)^2 \times 500 = 21$ multiply-branched structures instead of the 46 we observed. Similar considerations apply when other molecular forms were used as substrate. The large number of multiply-branched structures formed suggests that two or more branches were generated as part of a single exchange event. Furthermore, multiple branches were often structurally very close together.

When linear SV40 DNA was used as substrate, a considerable number of branched structures had an H-like configuration. Figure 2a, b, c and d shows H-like and Y-like structures. When linear DNA was used, degradation due to exonuclease(s) activity in the fraction did not always enable us to recover intact molecules; therefore we could not map the cross bar of the H-like structures relative to the extremities.

The distribution of lengths of the regions between two

Table 2 Summary of electron microscopic analysis of branched DNA structures

DNA	Branched (%)	Multiply-branched		No. molecules scored
		Branched	× 100	
Supercoiled	5.6	22.2		1,280
Open circles	23.8	47.6		500
Relaxed circles	20.6	46.1		500
Linear*	20.5	45.2		500

The various forms of SV40 DNA prepared as previously described^{1,4} were incubated with the phosphocellulose fractions in the conditions indicated in Fig. 1 at 10 µg ml⁻¹ for 2 h at 30 °C. Samples were prepared for electron microscopy as previously described.

*Various size fragments were produced when linear molecules were used as substrate. The percentage of branched molecules was estimated taking into account only molecules equal to or larger than SV40 full length size.

branched points were measured. The lengths varied from an apparent 0 to 0.5 fractional length, with an average of 0.15. Because SV40 chromosome contains about 5.1 Kb pairs, the average length of the region between two branched points corresponds to 765 nucleotides.

Models for the formation of branched DNA supported by evidence in the phage T4 and phage T7 experimental systems^{2,3}, suggest that H-like and Y-like structures could be formed by a process involving an exonuclease which initiates hydrolysis from a nick produced by an endonuclease of the type which we have described⁴. An expanded single-strand region which paired with DNA homologous in that region, would yield H-like structures. This would occur if breaks are introduced on the opposite strands of two paired molecules. Gapped molecules were frequently observed in the electron micrographs of DNA incubated with the fractionated extract (Fig. 2e and f). Y-like structures could originate from the H-like structures through branch migration or from recombination of a terminal unpaired region with an internal site of a gapped molecule.

Branched DNA structures could well be intermediates in the formation of complex DNA. A recombinant structure of two circular gapped molecules indistinguishable by electron microscopy from a late Cairns' replicative intermediate, could generate the various forms of complex DNA (Fig. 3).

H-like structures cannot give rise, by simple maturation of intermediates, to two reciprocal recombinants; our cell-free system is not faithfully mimicking meiotic recombination.

Fig. 2 Electron micrographs of branched structures obtained by incubating a phosphocellulose fraction with linear SV40 DNA produced by *EcoRI*. Supercoiled SV40 DNA was digested with *EcoRI* in a reaction mixture containing 100 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM MgCl₂ for 2 h at 37 °C. The reaction was stopped by addition of 1% sodium dodecyl sulphate and 15 mM EDTA and the linear DNA was purified on a 5–20% neutral sucrose gradient. The fractions corresponding to material sedimenting at 14.5S were pooled, precipitated with ethanol and stored at –20 °C. Sucrose gradients contained 0.1 M NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM EDTA, pH 8.0. (x 6,100). a and b, H-type recombinants; c and d, Y-type recombinants; e and f, gapped molecules. Arrows indicate gaps.

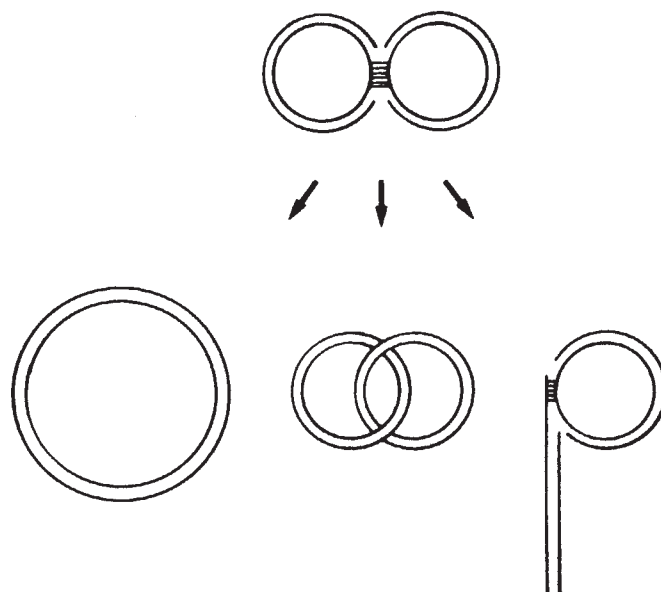
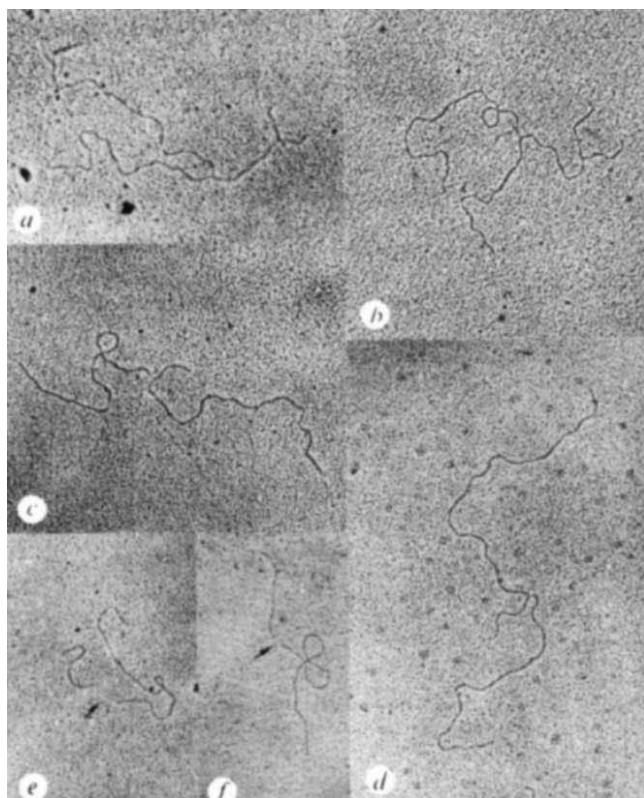


Fig. 3 Schematic representation of DNA recombination involving two gapped circles as intermediates. Dimers, catenated dimers and circles with tail are shown as possible final products.

In some systems recombination is brought about by a set of proteins operating in a complex⁵; if, however, some proteins are inactive or absent, structures which are not intermediates in the normal pathway could be formed.

It is possible that a type of recombination occurs in *Xenopus laevis* oocytes that produces a single recombinant chromosome. A circle of amplified ribosomal DNA could recombine with chromosomal DNA, and, by branch migration, roll along the chromosomal sequences, forming heteroduplex regions. Such structures could be responsible for rectification of sequences by gene conversion⁶, rectified chromosomal DNA being the final product.

The *in vivo* roles of the enzymes which produce the recombinant structures we have described remain unknown. But whatever the function of the enzymes, our system can produce heteroduplexes and can be used to investigate gene conversion in purified cell extracts.

After our manuscript was submitted for publication Benbow and Krauss reported the formation of recombinant DNA in a cell-free system of *Xenopus laevis* eggs⁷.

DOMENICA GANDINI ATTARDI

EMILIO MATTOCCIA

GLAUCO P. TOCCHINI-VALENTINI

Laboratory of Cell Biology,
Consiglio Nazionale delle Ricerche,
Via Romagnosi 18A,
Rome, Italy

Received 19 August; accepted 16 November 1977.

1. Gandini Attardi, D., Martini, G., Mattoccia, E. & Tocchini-Valentini, G. P. *Proc. natn. Acad. Sci. U.S.A.* 73, 554–558 (1976).
2. Broker, T. R. & Lehman, I. R. *J. molec. Biol.* 60, 131–149 (1971).
3. Tsujimoto, Y. & Ogawa, H. *J. molec. Biol.* 109, 423–436 (1977).
4. Mattoccia, E., Gandini Attardi, D. & Tocchini-Valentini, G. P. *Proc. natn. Acad. Sci. U.S.A.* 73, 4551–4554 (1976).
5. Mosig, G., Dannenberg, R. & Breschkin, A. *Indian J. Microbiol.* 15, 145–160 (1975).
6. Tartof, K. D. *A. Rev. Genet.* 9, 355–385 (1975).
7. Benbow, R. M. & Krauss, M. R. *Cell* 12, 191–204 (1977).

Nucleotide sequence of the attachment site of coliphage lambda

MANY bacteriophages and animal viruses, as well as DNA insertion elements and transposable antibiotic resistance factors, are capable of integrating their genomes into specific sites in the genome of their hosts, giving rise to a range of important biological phenomena¹⁻³. This site-specific recombination reaction has been studied most extensively in bacteriophage λ (ref. 3). During the process of lysogenisation the circularised phage DNA molecule is integrated into the *Escherichia coli* chromosome by reciprocal exchange between a specific site in the phage DNA molecule and a specific site in the *E. coli* chromosome between the *gal* and *bio* operons (Fig. 1). These sites are known as attachment sites (*att*). The reaction is catalysed by integrase, the product of the *int* gene^{4,5}. Excision of the prophage by site-specific recombination between the prophage end attachment sites require the action of the product of the *xis* gene as well as that of the integrase^{6,7}. In order to understand the mechanism of site-specific recombination it is necessary to study the DNA-protein interactions involved. Progress has recently been made in purification and study of the integrase⁸⁻¹⁰, and *in vitro* recombination systems have been developed^{11,12}. We report here an important step in our comprehension of the reaction at the molecular level, the determination of the DNA sequence of the λ attachment site.

The middle of the attachment site was mapped within *Hinf*I fragment 2, the central *Hinf*I fragment in the region between *Hind*III cut 3¹³ and *Bam*HI cut 3¹⁴, (Fig. 2), by Schreier *et al.*¹⁵.

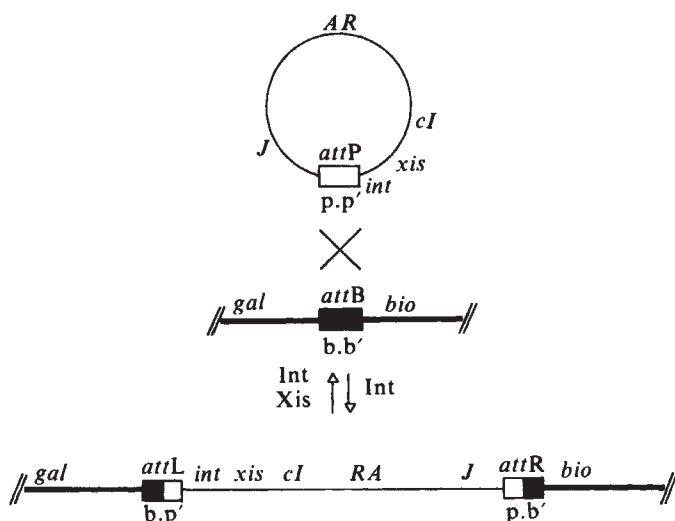


Fig. 1 Integration and excision of bacteriophage λ . Light lines represent the phage and prophage chromosome, heavy lines the bacterial chromosome. Rectangles represent the attachment sites. Genetic and physical data³ show that each attachment site is made up of nonhomologous 'recognition elements' bracketing a central crossover point or region. Thus the phage attachment site, *attP*, is shown formally as *p.p'*, the bacterial *attB* as *b.b'*. Distances between markers are arbitrary.

We have therefore determined the nucleotide sequence of *Hinf*I fragment 2 using the methods of Sanger and Coulson¹⁶ and Maxam and Gilbert¹⁷. Using *Hinf*I fragment 1 (Fig. 2) as a primer for incorporation with DNA polymerase I on a λ r-strand template the plus and minus method gave a sequence extending from 14 to 230 base pairs from *Hinf*I cut 1 to the right, and using *Hinf*I fragment 3 on a λ l-strand template we obtained a sequence extending from 300 to 210 base pairs from *Hinf*I cut 1 to the right.

The end sequences (base pairs 3-45 at the left end and 246-317 at the right end) were determined by separating the strands of 5'-end-labelled *Hinf*I fragment 2 and applying the method of Maxam and Gilbert¹⁷. Results obtained by the two methods agreed precisely. Figures 3 and 4 show plus and minus sequencing gels illustrating the central portion and the right half of *att* λ . The sequence that we have determined is shown in Fig. 5. This sequence differs from

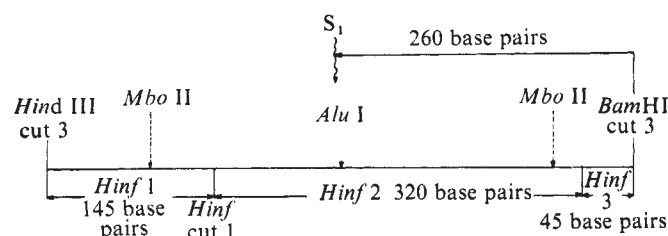


Fig. 2 Physical map of the *Hind*III cut 3-*Bam*HI cut 3 fragment containing the λ attachment site. The sizes of *Hinf*I fragments 1 and 3 are given in base pairs, and are based on the data of Schreier *et al.*¹⁵ and on the position of the labelled fragments in plus-minus sequencing gels when excess primer was used. The size of *Hinf*I fragment 2 is that determined by sequence analysis and the *Mbo*II cuts are placed accordingly. The solid vertical arrow above the *Alu*I site marks the position of the crossover point or left end of a common core (see text) as defined by the *S*₁ end of the *S*₁-*Bam*HI fragment¹⁵. The map positions of the *Hind*III and *Bam*HI cuts are taken from references 13 and 14.

that determined independently by Landy and Ross²⁶ only in having A in the l-strand in positions 134, 151 and 179, where they give T.

We can locate the crossover site within the attachment sequence at 105 ± 10 base pairs to the right of *Hinf*I cut 1. Schreier *et al.*¹⁵ used the fact that the junction of phage and bacterial DNA in hybrid prophage end attachment sites corresponds to the position of the crossover site. Transducing phages such as λ *pgal*₈ carry a prophage end *att*, so that digestion of heteroduplex molecules between transducing phages and wildtype λ with the single-strand-specific nuclease *S*₁ and a restriction endonuclease leaves a fragment with one end at the crossover site and the other at a known restriction site. The distance of the crossover site from the restriction site was determined by comparing the mobility of these fragments with the mobility of standard Φ X 174 fragments in polyacrylamide gels. The size of the relevant *S*₁-*Bam*HI fragment from λ /*pgal*₈ heteroduplex molecules is 260 base pairs both in the native and denatured state. Thus the crossover site maps 260 base pairs left of *Bam*HI cut 3¹⁵. These considerations apply to the simplest model of site-specific recombination, where the recombination event takes place at a single internucleotide position²⁰. An alternative model of *att* structure^{21,22} proposes that recombination does not occur at a single site but by means of staggered nicks left and right of a small central region of homology common to phage and bacterial *att*. In this case the left end of the common homologous region maps 260 base pairs left of *Bam*HI cut 3 since the common homologous region would be included within the *S*₁ fragment.

In Fig. 5 the position of the crossover site is given with respect to the nearest *Hinf*I cuts, which depends on measuring the size of the three *Hinf*I fragments shown in Fig. 2. *Hinf*I fragments 1 and 3 are consistently 145 and 45 base pairs long respectively from their mobility as native and denatured fragments. The sequence data given here define the length of *Hinf*I fragment 2 as 320 base pairs. Therefore the crossover site is $260 - 45 = 215$ base pairs left of *Hinf*I cut 2 (Fig. 2), and $320 - 215 = 105$ base pairs to the right of *Hinf*I cut 1. The error of this determination is ± 10 base pairs.

The results of previous investigations²²⁻²⁵ suggest that the attachment site consists of a central small crossover region flanked by 'recognition regions' that are not homologous with one another. Our sequence data, and that of Landy and Ross²⁶, fit in with this. Landy and Ross²⁶ find that the sequence from base

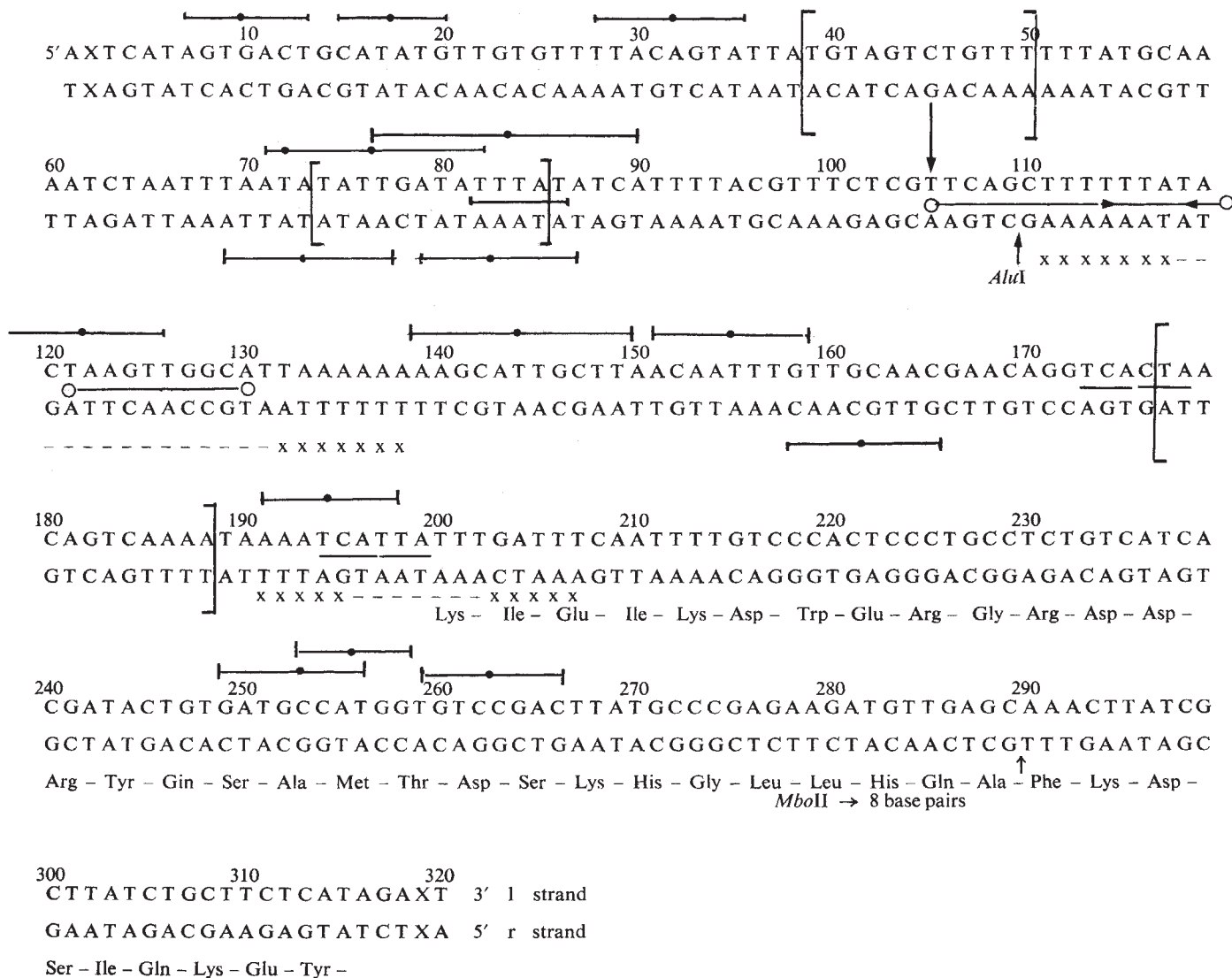


Fig. 5 Nucleotide sequence of *Hinfl* fragment 2 including the attachment sequence. The sequence was determined largely by the plus and minus sequencing method and partially by the method of Maxam and Gilbert as described in the text. To apply the latter method the strands of 5' end-labelled, denatured *Hinfl* fragment 2 were separated in a 6% polyacrylamide gel, extracted and treated as described by Maxam and Gilbert¹⁷. The very last nucleotides at each end were not detected so that the positions of the ends of the fragment are estimated from the known behaviour of marker dyes in the gels used. The sequence of the region from base pair 45 to 210 relies on priming in one direction only, but data from at least two gels for all parts of this region were in good agreement, and the plus and minus systems both gave the same sequence. The only places in the sequence where an alternative interpretation is possible are positions 6 and 13 where C instead of T is possible in the l-strand in both cases. In the region between base pairs 205 and 215 the control gratitudes were very faint, and the length of the runs was determined by measurement. The position of the S_1 end of the λ/λ pgal₈ S_1 -*Bam* HI fragment is shown by a solid arrow, and defines the crossover point or the left end of the common core (± 10 base pairs). Restriction sites in the sequence are indicated, and possible termination codons for the *int* gene are underlined. Sequences similar to recognition sites for RNA polymerase are in brackets. Palindromes are indicated by $\bullet\bullet$, direct repeats by $\blacktriangle\blacktriangle$ and inverted repeats by $x\ x\ x\ x$. Inverted repeats with three or fewer bases separating the two arms are classed as palindromes. $\circ\text{---}\circ$ indicates sequences related to sequences found in the inverted repeat at the end of IS1 (N. Grindley, personal communication).

121 to 130 is identical to an ISI sequence, and the sequence from base pair 105 to 119 has some similarity to the same part of the ISI sequence in the other orientation. The functional significance of these sequences and of those related to promoter sequences remains to be shown.

The DNA sequence presented here provides the basis for further definition of important features of DNA sequences involved in site-specific recombination.

We thank F. Sanger for laboratory facilities, J. Fiddes for introducing us to the plus and minus technique, A. Maxam and W. Gilbert for sending pre-publication details of their sequencing method and B. Müller-Hill for encouragement. This work was supported by a grant from the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 74 to R.W.D. and by a

Graduiertenstipendium des Landes Nordrhein-Westfalen to P.H.S.

R. WAYNE DAVIES*
P. H. SCHREIER
D. E. BÜCHEL

Institut für Genetik,
Universität zu Köln,
Weyertal 121,
D 500 Köln 41, FRG

Received 20 July; accepted 7 November 1977.

*Present address: Department of Biology, University of Essex, Wivenhoe Park, Colchester, UK.

- Cohen, S. N. *Nature* **263**, 731–738 (1976).
- Battula, N. & Temin, H. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 281–285 (1977).
- Gottesman, M. E. & Weissberg, R. A. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 113–138 (Cold Spring Harbor Laboratory, New York, 1971).
- Zissler, J. *Virology* **31**, 189 (1967).
- Gingery, R. & Echols, H. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1507–1514 (1969).
- Guarneros, G. & Echols, H. *J. molec. Biol.* **47**, 565–574 (1970).
- Kaiser, A. D. & Masuda, Y. *J. molec. Biol.* **47**, 557–564 (1970).
- Nash, H. A. *Nature* **247**, 543–545 (1974).
- Kotewicz, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 1511–1515 (1977).
- Enquist, L. W. & Weissberg, R. A. *J. molec. Biol.* **111**, 97–120 (1977).
- Gottesman, S. & Gottesman, M. E. *J. molec. Biol.* **81**, 489–499 (1975).
- Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1072–1076 (1975).
- Murray, K. & Murray, N. E. *J. molec. Biol.* **98**, 551–564 (1975).
- Haggerty, D. M. & Schleif, R. F. *J. Virol.* **18**, 659–663 (1976).
- Schreier, P. H. *et al.* *Nature* **267**, 555–557 (1977).
- Sanger, F. & Coulson, A. R. *J. molec. Biol.* **94**, 441–448 (1975).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
- Wilson, G. A. & Young, F. E. *J. molec. Biol.* **97**, 123–125 (1975).
- Hradecna, Z. & Szybalski, W. *Virology* **32**, 633–643 (1967).
- Dove, W. F. *J. molec. Biol.* **47**, 585–589 (1970).
- Signer, E. A. *Rev. Microbiol.* **22**, 451–488 (1968).
- Shulman, M. & Gottesman, M. E. *J. molec. Biol.* **81**, 461–482 (1973).
- Guerrini, F. *J. molec. Biol.* **46**, 523–542 (1969).
- Davis, R. W. & Parkinson, J. *J. molec. Biol.* **56**, 403–423 (1971).
- Shulman, M., Mizuuchi, K. & Gottesman, M. E. *Virology* **72**, 13–22 (1976).
- Landy, A. & Ross, W. *Science* **197**, 1147–1160 (1977).
- Davidson, N. & Szybalski, W. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 45–82 (Cold Spring Harbor Laboratory, New York, 1971).
- Botchan, P. *J. molec. Biol.* **105**, 161–176 (1976).
- Mizuuchi, K. & Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3524–3528 (1976).
- Gellert, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4474–4478 (1976).
- Pribnow, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 784–788 (1975).

Plant viruses with circular single-stranded DNA

SMALL quasi-isometric particles, mostly occurring in pairs, have been found in, and purified from extracts of plants infected by maize streak¹, beet curly top², tomato golden mosaic³, euphorbia mosaic³, bean golden (yellow) mosaic^{3–5}, cassava latent⁶ and cassava brown streak viruses⁶. Individual particles are 15–20 nm in diameter—unusually small for a virus—and in electron micrographs many of the individual particles in the pairs have a five-sided outline in which the contiguous sides seem longer than the others. The pairs of particles of maize streak and cassava latent viruses have sedimentation coefficients of about 76S (refs 1 and 7) and preparations of each yield a single polypeptide species, estimated at about 28,000 and 34,000 daltons respectively⁷. Their nucleic acid can be resolved into two components by electrophoresis⁷, and in maize streak virus it has been identified tentatively as RNA on the basis of sensitivity to ribonuclease¹. We now report, however, that the nucleic acids of cassava latent and maize streak viruses consist of single-stranded, predominantly circular DNA of molecular weight less than 10⁶.

Maize streak virus was purified as before¹ from maize plants infected by the leafhopper *Cicadulina mbila*. Cassava latent virus was purified from systemically infected leaves of *Nicotiana glauca* plants that were inoculated manually with sap. Some preparations were purified as before⁷, but in most purifications formaldehyde was omitted from the buffer used to resuspend the sediments of the first high-speed centrifugation. Viruses were propagated and purified at Nairobi, and nucleic acid was extracted there for infectivity tests. Nucleic acid for analysis was extracted at Invergowrie and examined by electron microscopy at Glasgow.

Electron microscopy showed that virus preparations contained many isometric particles of characteristic size, mostly in pairs. Maize streak virus particles seemed better preserved than those of cassava latent virus. When extracted¹⁷ and analysed on 2.2 % polyacrylamide gels in our standard electrophoresis buffer (0.036 M Tris, 0.03 M NaH₂PO₄, 0.001 M EDTA, pH 7.8), the nucleic acid of each virus usually gave two well resolved peaks (Fig. 1), but sometimes a single peak with a shoulder was obtained, as found before with that buffer⁷. There was usually more of the slower migrating component. Peaks were unaffected when samples were treated with briefly boiled RNase A before electrophoresis but were absent after

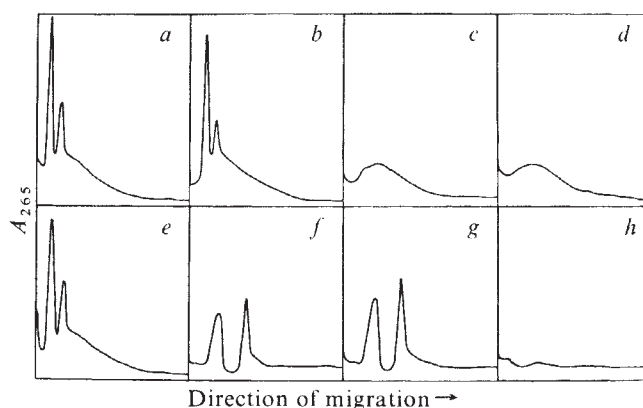


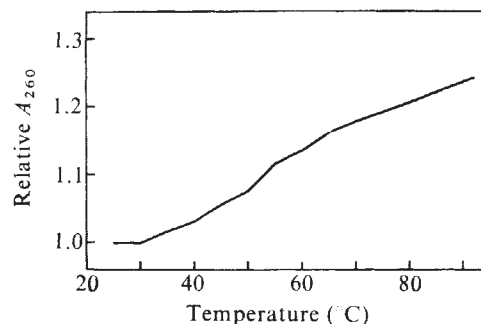
Fig. 1 Effects of enzyme treatments on virus nucleic acid species separated by electrophoresis on 2.2% polyacrylamide gels. Nucleic acid (25–50 µg ml⁻¹) was extracted using Pronase–sodium dodecyl sulphate¹⁷ from cassava latent virus purified as described by Bock *et al.*⁷ (a,e), cassava latent virus purified by the usual method (b–d), and maize streak virus (f–h). Treatments, for 30 min at 37 °C, were in 0.05 M Tris buffer, pH 7.5 (a–c and e–h) or in 0.01 M sodium acetate buffer, pH 5.0, containing 0.1 M sodium chloride (d). a, b, f, Buffer controls; c, h, DNase I (1 µg ml⁻¹ DN-EP, Sigma) + 3 mM MgCl₂; d, S1 nuclease (500 U ml⁻¹ Type III, Sigma); e, g, RNase A (1 µg ml⁻¹, bovine pancreatic, Type IA, Sigma, previously boiled for 1 min). After treatment, 4 volumes of ethanol were added to each sample. After resuspending the precipitates in standard electrophoresis buffer containing 8 M urea, the samples were heated for 15 min at 60 °C and electrophoresed for 3–3.5 h¹⁷. The gels were washed for 16 h in water and examined using an ultraviolet Scanner (Joyce Loebel).

treatment with DNase I (Fig. 1). Moreover they were unaffected by treating cassava latent virus nucleic acid with alkali (0.3 M NaOH for 8 h at 37 °C) before electrophoresis. The ability of cassava latent virus nucleic acid to infect *N. glauca* was destroyed by DNase but not RNase. Thus the nucleic acid of both viruses is DNA. The tentative identification of the nucleic acid of maize streak virus as RNA¹ can be attributed to the use of unboiled ribonuclease, presumably contaminated with DNase.

When nucleic acid of cassava latent virus was exposed to nuclease S1 before electrophoresis, neither of the characteristic nucleic acid species was found (Fig. 1), indicating that they are largely or entirely single-stranded. Resistance to the three nucleases was the same for nucleic acid from cassava latent virus purified with formaldehyde as when no formaldehyde was used.

Further evidence that cassava latent virus nucleic acid is not substantially double-stranded came from plots of ultraviolet

Fig. 2 Effect of temperature on ultraviolet absorption of cassava latent virus nucleic acid. Sample in 1.5 mM sodium chloride, 0.15 mM sodium citrate was heated at 0.5 °C per min. Readings are corrected for sample expansion.



a

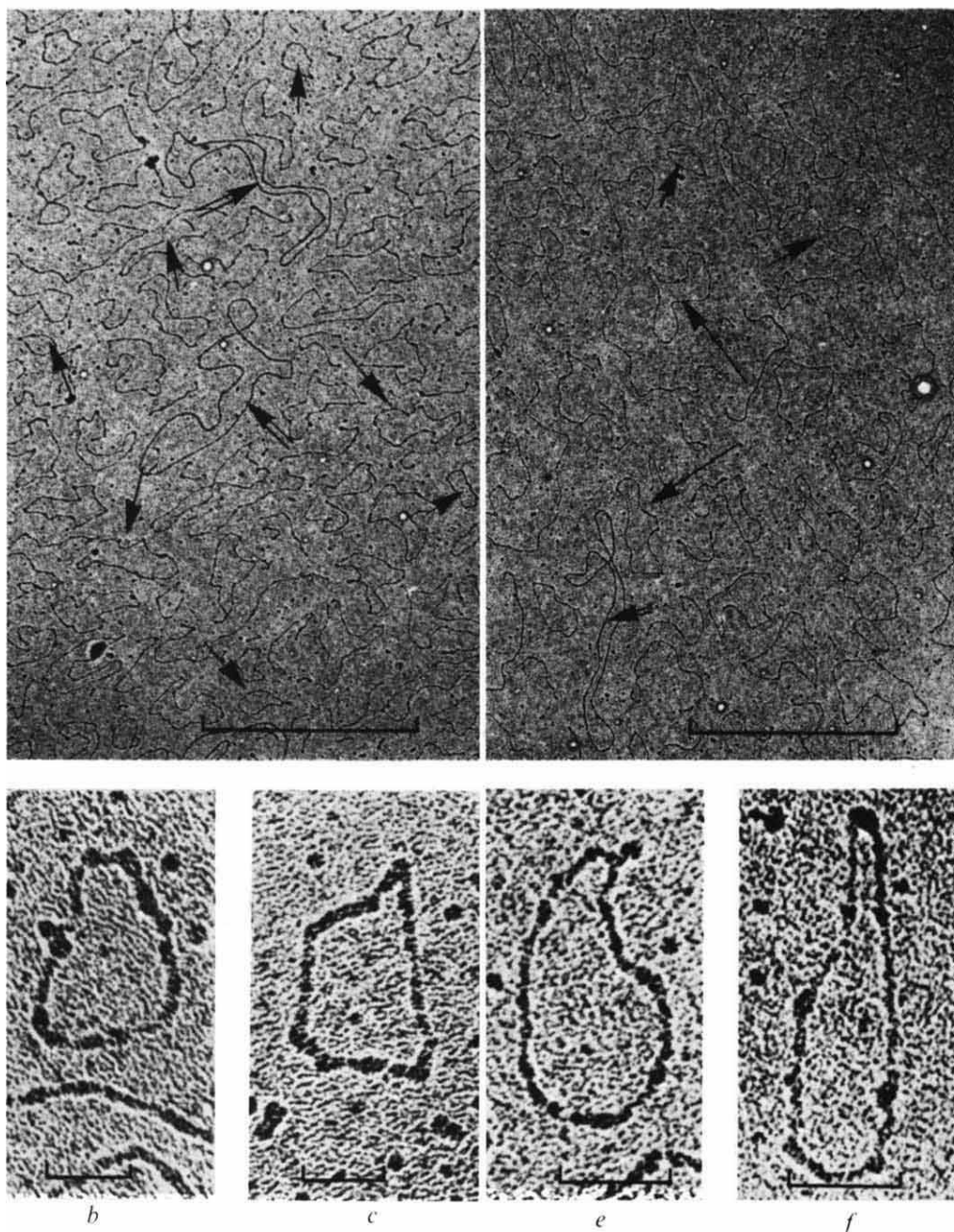


Fig. 3 Electron micrographs of nucleic acid molecules of maize streak (*a*, *b* and *c*), and cassava latent (*d*, *e* and *f*) viruses. The small circles in (*a*) and (*d*) are the plant virus nucleic acid molecules (short arrows), the middle-size circles are phage Φ X174 strain am3 single-stranded DNA (long arrows) and the large circles are phage PM2 double-stranded DNA (double-tailed arrows). The bar represents 1 μ m. Individual molecules of maize streak virus nucleic acid are illustrated at higher magnification in (*b*) and (*c*) and those of cassava latent virus nucleic acid in (*e*) and (*f*). The bar represents 0.1 μ m. The nucleic acid was prepared for electron microscopy by the formamide modification of the protein film technique (ref. 12) from 50% (v/v) formamide, 0.1 M Tris-HCl, 0.01 M EDTA (pH 8.5) on to a hypophase of 20% (v/v) formamide, 0.01 M Tris-HCl, 0.001 M EDTA (pH 8.7). Micrographs were taken with a Siemens 101 electron microscope at 40 kV and a nominal magnification of 12,000 fold.

absorption at 260 nm in the range of 25°–92 °C (Fig. 2). There was no evidence of cooperative melting. Moreover when the nucleic acids were heated to 92 °C, cooled slowly to 25 °C and heated again to 90 °C, the final absorbance curve was essentially the same shape as before, indicating that no extensive double-stranded structures had formed on cooling, and excluding the possibility that some virus particles contain DNA-plus strands while a substantial proportion contain complementary minus strands instead, as with some parvoviruses⁹.

Electron microscopy of preparations of nucleic acid of either virus revealed two sorts of molecule (Figs 3 and 4). Small circular molecules predominated in most preparations but in some there were about as many linear as circular molecules and, in a few, linear molecules predominated. Linear molecules varied in size up to the length of the circular molecules (Fig. 4), suggesting that they are formed by nicking and partial degradation of the latter. In samples to which double-stranded circular DNA of phage PM2¹⁰ and single-stranded circular DNA of phage Φ X174 (strain am3)¹¹ were added, the plant virus DNA molecules

appeared single-stranded throughout.

Molecular weight was estimated from relative molecule lengths¹². Comparison of modal lengths showed that the circular DNA of cassava latent virus was about 50% of the size of phage Φ X174-DNA (strain am3; 1.59×10^6 daltons^{11,13}), and had a molecular weight of $0.80 \pm 0.01 \times 10^6$ (Fig. 5). The figures for maize streak virus DNA were about 45% and $0.71 \pm 0.01 \times 10^6$.

Length-distribution diagrams of DNA molecules from each virus (Fig. 4) suggested that the two nucleic acid species detected by polyacrylamide gel electrophoresis are linear and circular molecules of the same length. To examine this further, the two species in a preparation of cassava latent virus DNA were separated by polyacrylamide gel electrophoresis for 13 h, and the nucleic acid was extracted from each band by steeping the disrupted gel slices for 4–5 h at 4 °C in 2 ml 50% (v/v) formamide containing 1.5 mM sodium chloride and 0.15 mM sodium citrate. After low-speed centrifugation to remove gel fragments, the nucleic acid was concentrated from the super-

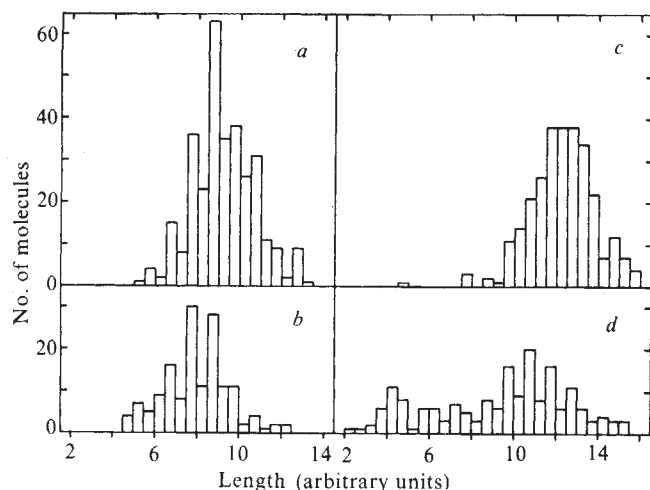


Fig. 4 Relative lengths of circular and linear molecules of virus DNA. *a*, Maize streak virus circular DNA (314 molecules); *b*, maize streak virus linear DNA (151 molecules); *c*, cassava latent virus circular DNA (279 molecules); *d*, cassava latent virus linear DNA (179 molecules). The molecules were measured with a map ruler from electron micrographs enlarged 25-fold.

nant fluid by precipitation with 4 volumes of ethanol, and the two samples were examined by electron microscopy. The sample of faster migrating component contained linear molecules only, many of similar length to the circular molecules. More than

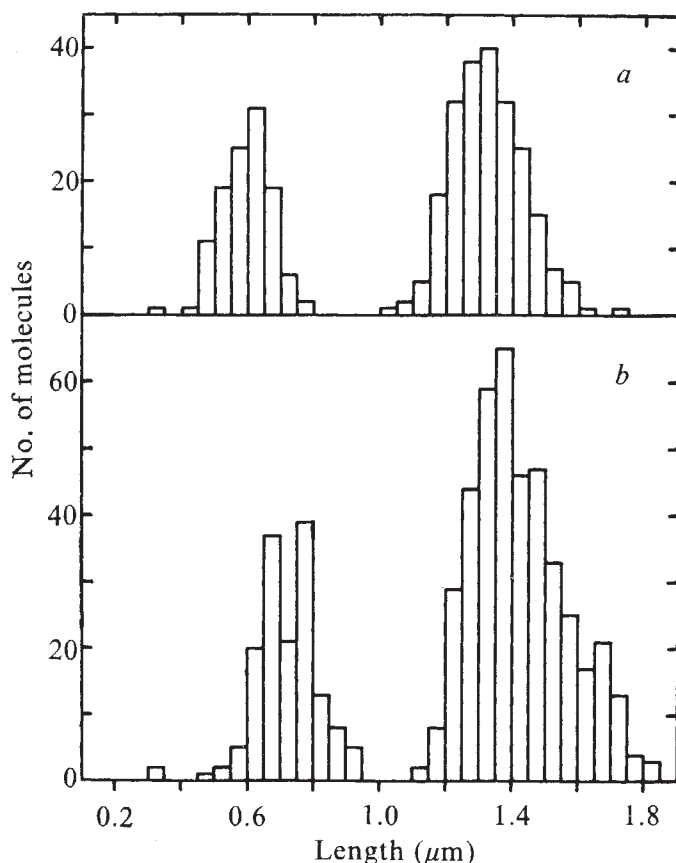


Fig. 5 Length-distribution diagrams for circular DNA molecules. *a*, Maize streak virus (115 molecules) and ΦX174-am3 (222 molecules); *b*, cassava latent virus (153 molecules) and Φ174-am3 (416 molecules). Each pair of DNA samples was mixed and spread. The method of specimen preparation is described in Fig. 3. Lengths were measured with a computer-assisted planimetric device¹⁸ from micrographs enlarged 10-fold, the circles of ΦX174 single-stranded DNA being used as an internal standard.

80% of molecules in the sample of slower migrating component, however, were circular. We conclude that the circular and linear molecules are respectively the slower and faster migrating component in polyacrylamide gels.

Working with single-stranded DNA from SV40 virus, Dingman *et al.*¹⁴ found that the relative speeds of electrophoretic migration of circular and linear molecules of the same length depended on the concentration of the gel. Hence we attribute differences in the separation of the two DNA species in our gels to small uncontrolled differences in concentration of different batches of gel.

It seems likely that the nucleic acids of other plant viruses with small paired particles will be found to have similar single-stranded DNA. Of such viruses, some have leafhopper vectors whereas others have whitefly vectors. Maize streak virus is a representative of the first type¹⁵; the vector of cassava latent virus is not known. However, bean golden yellow mosaic virus has a whitefly vector, and also contains single-stranded DNA, of estimated molecular weight $0.66\text{--}0.95 \times 10^6$ (refs 8 and 16). We see in this and other similarities the emergence of a new virus group, comprising the plant viruses with small paired particles. The name geminivirus is proposed for this group, which will be described in detail elsewhere (unpublished data of K. R. B.).

Our work has provided no support for the suggestion⁷ that the genome of maize streak and cassava latent viruses is in two parts contained respectively in the two particles that make up a pair. If, as we suggest, one of the nucleic acid species separated by gel electrophoresis is derived from the other, the DNA genomes would seem the smallest known for independently replicating viruses.

We thank Drs B. A. Allsopp and D. J. Robinson for help and advice; Miss S. Barr for technical assistance; Drs A. Campbell and R. Eason for PM2 DNA and ΦX174 DNA, respectively; Drs H. Y. Elder and V. A. Moss for the use of the planimeter; Mr I. M. Roberts and Mrs A. M. Hutcheson for electron microscopy; Dr J. Hay for advice, and Dr R. M. Goodman for sending his manuscript before publication.

B. D. HARRISON
H. BARKER

Scottish Horticultural Research Institute,
Invergowrie, Dundee, UK

K. R. BOCK
E. J. GUTHRIE
GINA MEREDITH

Ministry of Overseas Development Crop Virology Project,
East African Agriculture and Forestry Research Organisation,
PO Box 30148, Nairobi, Kenya

M. ATKINSON

MRC Virology Unit,
Institute of Virology,
Church St, Glasgow, UK

Received 27 July; accepted 4 November 1977.

1. Bock, K. R., Guthrie, E. J. & Woods, R. D. *Ann. appl. Biol.* 77, 289–296 (1974).
2. Mumford, D. L. *Phytopathology* 64, 136–139 (1974).
3. Matys, J. C., Silva, D. M., Oliveira, A. R. & Costa, A. S. *Sunna Phytopathologica* 1, 267–274 (1975).
4. Galvez, G. E. & Castano, M. *Turrialba* 26, 205–207 (1976).
5. Goodman, R. M., Bird, J. & Thongmeekarn, P. *Phytopathology* 67, 37–42 (1977).
6. Bock, K. R. & Guthrie, E. J. in *African Cassava Mosaic* (ed. Nestel, B. L.) 11–16 (International Development Research Centre, Ottawa, 1976).
7. Bock, K. R., Guthrie, E. J., Meredith, G. & Barker, H. *Ann. appl. Biol.* 85, 305–308 (1977).
8. Goodman, R. M. *Nature* 266, 54–55 (1977).
9. Rose, J. A., Berns, K. I., Hoggan, M. D. & Koczot, F. J. *Proc. natn. Acad. Sci. U.S.A.* 64, 863–869 (1969).
10. Espejo, R. T., Canelo, E. S. & Sinsheimer, R. L. *Proc. natn. Acad. Sci. U.S.A.* 63, 1164–1168 (1969).
11. Zuccarelli, A. J., Benbow, R. M. & Sinsheimer, R. L. *Proc. natn. Acad. Sci. U.S.A.* 69, 1905–1910 (1972).
12. Davis, R. W., Simon, M. & Davidson, N. *Methods in Enzymology* (eds Grossman, L. & Moldave, K.) 21D, 413–428 (Academic Press, New York and London, 1971).
13. Sinsheimer, R. L. *J. mol. Biol.* 1, 43–53 (1959).
14. Dingman, C. W., Fisher, M. P. & Kakefuda, T. *Biochemistry* 11, 1242–1250 (1972).
15. Storey, H. H. *Ann. appl. Biol.* 12, 422–439 (1925).
16. Goodman, R. M. *Virology* 83, 171–179 (1977).
17. Murrant, A. F., Mayo, M. A., Harrison, B. D. & Goold, R. A. *J. gen. Virol.* 16, 327–338 (1972).
18. Biddlecombe, W. H. *et al. J. Physiol.* (in the press).

reviews

Animal signal characteristics

P. J. B. Slater

Optical Signals: Animal Communication and Light. By Jack P. Hailman. Pp. xix + 362 (Indiana University: Bloomington, Indiana and London, 1977.) £11.25. *Animal Communication.* Second edition. By Hubert and Mable Frings. Pp. ix + 207. (University of Oklahoma: Norman, Oklahoma, 1977.) Hardback \$9.95; paperback \$4.95.

THE striking and often stereotyped displays with which animals communicate have always fascinated ethologists. The similarity of signals within a species, and the differences between species, make them well suited to the study of behavioural evolution. By examining the contexts in which signals occur, some progress has also been made towards understanding the motivational systems underlying them and thus the messages which they convey. On the other hand, the selective forces which have led them to be as they are have received less attention.

It is on this last area, the ways in which signals are adapted to the environments in which they occur and to the functions which they have, that Hailman hopes to shed some light. He was originally asked to write a chapter on visual signals for a book to be edited by Sebeok but, having over-run his word limit, he decided to use the additional material for a book of his own. His aim in this is a limited one: "to specify optical principles, search for ecologically relevant situations, and then predict animal signal characteristics". As this is a largely unexplored area, the range of topics covered is unconventional for a book on animal communication.

The first two chapters outline the author's approach to the problem, dealing in particular with scientific philosophy and information theory. The following three are concerned with the properties of the channel, the sender and the receiver: in effect, optics, the basis of animal colours and movements, and visual sensation and perception. These chapters provide a wealth of background material covering every physical and physiological process which might conceivably be relevant to the form of animal signals. The rest of the book gets down to examining the sorts of signals which

should emerge, given these constraints. A chapter on deception, dealing largely with crypsis and mimicry, is followed by one on noise, in which the ways in which animals may achieve conspicuousness in various situations are explored. A further chapter deals with the sorts of signals which might carry different types of information.

The book is a bold attempt to take a novel approach to visual communication, but in many ways it is hindered by the very novelty of that approach. Although the background information in the earlier chapters is solid and well-established, the later ones sink into the haze which often surrounds functional questions. To approach such problems as "Why is the cardinal red?" one can only resort to correlational methods with all their attendant difficulties, looking across species to see if red colouration occurs in some situations more than in others. The correlations the author uses are neither extensive nor formal, so that many of his suggested explanations rest on speculations from selected data. He has, however, done a service by pointing to an inter-

esting and neglected area. If he has been unable to pull all the strings together himself, he has at least provided a basis on which research towards doing so may be founded.

Hailman's book is tough reading: it is tersely written and contains few detailed examples; the author has a liking for classifying things with the aid of a confusing variety of polysyllabic labels; the text is in typescript form and contains many misprints. Although also poorly produced (two pages are even printed in the wrong order), the book by Frings and Frings is something of a contrast, as it was written as a brief introduction for laymen, and contains many examples and little theory. Such theory as it does include is, however, seriously out of date, for it was originally published in 1964 and the new edition has been simply updated by adding a chapter on recent advances. This is an unhappy compromise which can hardly do justice to what has happened since then. □

P. J. B. Slater is Lecturer in Animal Behaviour at the University of Sussex, UK.

Lunar studies

The Moon: A New Appraisal from Space Missions and Laboratory Analyses. Edited by G. M. Brown, G. Eglinton, S. K. Runcorn and H. C. Urey. Pp. vi + 606 + 15 plates. (Royal Society: London, 1977.) £38 in the UK; £38.95 overseas.

THIS book is a collection of papers given at a Royal Society symposium in 1975. Many, perhaps most, of the leading research groups in lunar science are represented. It represents well the state of the subject at the time of presentation.

The climactic period of lunar studies began with the Apollo missions in 1969–72, but it did not end there. The rich collection of lunar samples returned by US and Soviet missions, the experiments left on the moon, and the data tapes obtained during the missions, have provided the basis for a vigorous and even an expanding science. Although the work has been published in all the appropriate journals (including *Nature*), it has a special point of concentration in the Proceedings of the Lunar Science Conferences held each year in Houston. The worker seeking direct access to some

aspect of lunar research should begin there.

What then is the role of the present volume? The editors have made a deliberate, and quite successful, effort to make the work accessible to a wider scientific public. Authors have been encouraged to begin at the beginning, and to set their work in context. As a result, some of the papers are admirable introductions to important and difficult subjects. Others are broad-brush extended abstracts, which can at least alert the reader to the significance of an area, or the point of view of one group. Narrower, more technical papers have not been eliminated, but they do not dominate the whole.

The first section, on the accumulation and bulk composition of the moon, is especially good. The evidence, mainly isotopic and chemical, is presented with care by Wassergburg, Anders, Wänke, and Lal *et al.* I know of no more accessible current summaries of the beautiful development of early lunar chronology, or of the striking patterns of trace element abundances.

The organisation of such a complex subject is always a problem. Certain articles in later sections are very close to this group, and should in my judgment be read with them. A later article by Palme and Wänke, one on lunar gravity by Sjogren, and three in the last section beginning with that of Kopal, are among these. They bear closely on questions of origin and early history.

The period of mare formation, extending roughly from 4×10^9 to 3×10^9 y ago, was an active stage about which we know a great deal. The least clear point is the relationship, causal and temporal, between the major impacts which produced huge basins on both the front and back sides of the planet, and the basaltic flows which created the maria on the side we see. This is a major theme of Section III; the articles by Geiss *et al.* and by O'Hara *et al.* deal especially with important aspects of the subject.

The meteoritic bombardment of the lunar surface continues to the present. It is important in its own right, and also as a chronological tool. A direct comparison of ages in different lunar regions is possible, and to some extent the chronology can be given an absolute basis, using the lunar samples. The comparison with other planets, especially Mercury and Mars, is more difficult. Articles in this section by Neukum and Housley, among others, are notable. Signer *et al.* call attention to some gaps in our understanding of lunar rare gases. Strangway and Olhoeft, and others, demonstrate the importance of electrical

measurements in understanding the interior. Coleman and Russell, Runcorn, and others, summarise our knowledge of the striking magnetic remanence seen in the lunar samples, and in the orbital data. The coverage of geophysical aspects of lunar science is in fact especially thorough.

Allowing for the fact that any symposium must contain some weak contributions along with the strong, this volume can be highly recommended. Its one general fault is shared with many others: it appears more than two years after the event. There have been remarkable developments since, which cannot be reflected here and must be found in the current literature. Chronological and isotopic studies, comparative lunar and meteoritic work, and the interpretation of orbital remote sensing data are among the areas that have moved ahead rapidly since 1975.

The distinguished editors have chosen to close the volume with an "Epilogue" showing a colour plate of Apollo orbital geochemical data, and pointing out the importance, for our understanding of the origin of the moon and the Solar System, of a global survey by a complete set of geochemical and geophysical instruments. This describes a potential future mission, the Lunar Polar Orbiter. Since this is a cause to which this reviewer has been passionately committed, he can hardly fail to agree with these sentiments.

James R. Arnold

James R. Arnold is Professor of Chemistry at the University of California at San Diego.

Charged particle beams

The Physics of Charged-Particle Beams. By J. D. Lawson. Pp. xxi+462. (Oxford University. (Clarendon): Oxford; 1977.) £16.50.

CHARGED PARTICLE BEAMS have served to probe the nucleus, to analyse minute quantities of material, to reproduce television images, to resolve living structures smaller than the wavelength of light, to cut and join metals, and to reveal the subnuclear universe, as tiny and unexpected as that of the astronomer is large and apparent. In the future, one hopes they will provide the means to contain and perhaps ignite fusion reactions and prove useful in the treatment of malignant disease.

The field is as diverse in its intellectual content as in its applications. Based firmly in classical electromagnetism, it extends to a mathematics of collective phenomena as fascinating as any to be found in the fields of pure science to which it has been applied.

Lawson is particularly well placed, having contributed to most branches of this field of applied physics, to write an authoritative and unifying text. This he does, manfully striding through six chapters of somewhat daunting mathematical equations, from some which the under-graduate will recognise to others which still puzzle the expert.

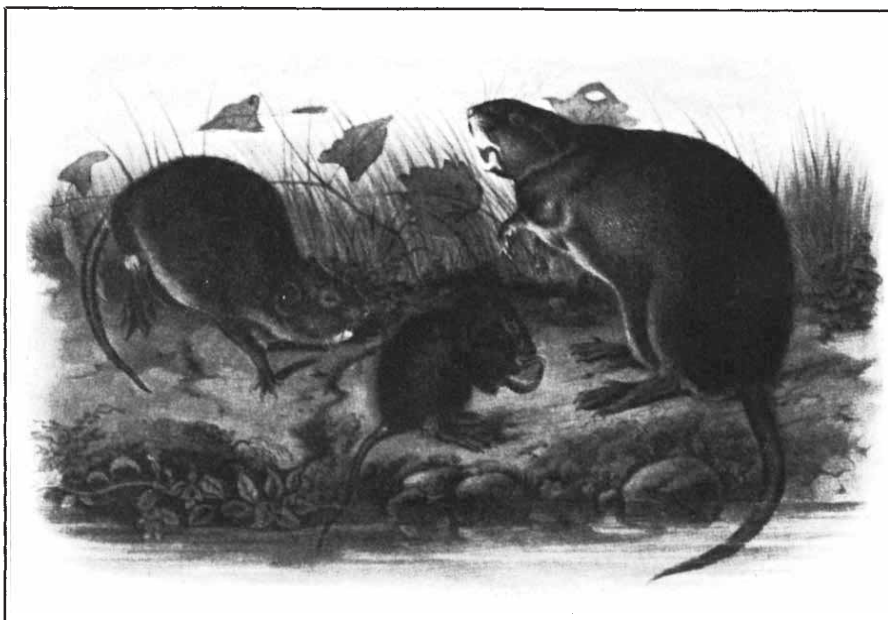
Besides the achievements of charged particle beam physics, the book stands as might a great master's treatise on perspective against the riches of renaissance art, perhaps somewhat disappointing to those who would appreciate but invaluable to those who would contribute.

The material is organised in an intellectual sequence which will frustrate those who may seek mathematical assistance in the solution of a particular problem. But no matter, it should be read from cover to cover, for Lawson disregards the partitions between specialities in the field in an attempt to cross pollinate neighbouring branches of the tree of physics whose blossoms, beautiful as they may be, might otherwise begin to show signs of infertility.

Such a book has been long awaited, particularly by those of us in the particle accelerator and fusion fields. It is regrettable that its printers have reverted to a typescript presentation that would have offended Gutenberg. Provided the reader can overcome his aesthetic scruples, however, he will find the layout of equations, figures, references and indexing perfectly adequate.

E. J. N. Wilson

E. J. N. Wilson is a senior physicist in the team which constructed the 400 GeV Super Proton Synchrotron at the European Organisation for Nuclear Research (CERN), Geneva.



Family of muskrats (*Ondatra zibethica*). By John James Audubon in his famous book *Quadrupeds of North America* (1849). Illustration taken from *Larousse Animal Portraits*, compiled by P. P. Grasse (Hamlyn: London, New York, Sidney and Toronto, 1977; £6.95). The book contains over eighty Natural History prints from the seventeenth to the twentieth century, a large proportion of which were selected from a collection in the Natural History Museum in Paris. The collection was started by a brother of Louis XIII, Gaston of Orleans, a keen naturalist and patron of the arts.

Experimental design and statistical analysis

Experiments: Design and Analysis. Second edition. By J. A. John and M. H. Quenouille. Pp. 296. (Charles Griffin: London and High Wycombe, 1977.) £12.

The Design and Analysis of Experiment by Quenouille, first published in 1953, was an early text which emphasised the practical aspects of experimental design and statistical analysis. The second edition, which appears under the joint authorship of J. A. John and Quenouille, who died at an early stage in its preparation, preserves the spirit of the original, with detailed coverage of the more widely used designs fully illustrated by numerical examples from either agricultural or animal experiments.

Despite the high standard of the original, the changes incorporated in the new edition are welcome; the grouping of chapters into sections has been abandoned and some re-arrangement has allowed the inclusion of two new chapters—one on fractional replication and the other on response surface methods—without increasing their total number. The contents have been updated with advances made since first publication, and strengthened with further elaboration of specific designs. The bibliography has been revised and expanded.

The first four chapters discuss randomised blocks, Latin squares, and factorial and split-plot designs. Techniques for missing-values, originally confined to a separate chapter, have been inserted at the appropriate places. Discussion of missing-values in randomised blocks is particularly extensive, although it is strange that two missing-values in such designs should be estimated by iteration rather than directly equating the appropriate residuals to zero.

The next four chapters illustrate the main modifications of full factorials through confounding and partial replication. The new chapter 9, on response surface methods, provides an excellent account of first- and second-order designs, including rotatability; examination of the fitted response surface is particularly well illustrated.

Chapter 10, on incomplete block designs, includes recent work on resolvable and cyclic designs and a brief introduction to partially-balanced incomplete blocks. The three chapters (11, 12 and 13) on designs for estimating residual effects in long-term experiments, planning of groups of ex-

periments and combination of results, still constitute one of the few adequate expositions of these important topics. The final chapter describes the scaling of data, and the book ends with tables of t , F , χ^2 , studentised range and random permutations, a bibliography and a good index.

This book is a valuable guide to the understanding of experimental design: its discussion of real data and the

absence of mathematical and statistical models should appeal to the experimenter. Its comprehensive coverage of the simpler designs and the tabular summaries of their properties provide a useful reference for the statistician.

A. L. Johnson

A. L. Johnson is a statistician in the MRC Statistical Research and Services Unit at University College Hospital Medical School, London, UK.

Population differentiation and gene-flow

Geographic Variation, Speciation, and Clines. By John A. Endler. Pp. ix+246. (Princeton University: Princeton, New Jersey, 1977.) Hardback £12; paperback £5.45.

CHARLES DARWIN, with his knack for picking a good problem, wrote: "Those forms which possess in some considerable degree the character of species, but . . . are so closely linked . . . by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us." Endler's book reviews the diverse and scattered literature on clines—that is, the study of population differentiation in the absence of barriers to gene-flow—that has accumulated since Darwin.

It directs itself to two fundamental questions. First, it asks whether sharp geographical differentiation can evolve across a spatially or genetically continuous series of populations; or whether gene-flow can prevent spatial differentiation. The answer is rather clear. Subspecific and even specific differentiation (parapatric speciation) in the absence of barriers to gene-flow is as common and important as divergence with strong barriers, whatever our introductory textbooks might say.

Second, it asks about the shape of clines; for example, whether stepped clines require stepped environments and whether there is always a unique one-to-one correspondence between local steepening and environmental change. Endler explores the rich possibilities in considerable detail, using analytical and computer models, as well as laboratory populations. The fairest summary is that "it depends". Chapter 4, in particular, shows how conclusions based on classical single-locus models can be much influenced by the effects of co-adapted modifiers.

Whereas chapters 2–4 consist of rather detailed population genetics, the last two chapters, which they underpin, are of more general interest. Chapter 5 elaborates the earlier con-

clusion that parapatric speciation is probably common. Indeed, present-day patterns of distribution resulting from parapatric speciation do not differ significantly from those generated by allopatric speciation and secondary contact. Even the familiar explanations for present-day distribution patterns of Amazonian and African forest birds, butterflies and other organisms, based on Pleistocene refugia within forest remnants and subsequent secondary contact, look shaky in the light of Endler's reanalysis in chapter 6. These distributions can equally well be explained by the presence of some kind of selection gradient, which may be sufficient to maintain geographical differentiation regardless of whether or not the forests have been fragmented.

The book summarises a great deal of empirical data on clines of all kinds, and makes a brave attempt to match theory with data wherever possible. Efforts to assign numbers to key parameters—for example, rates and distances of gene-flow and cline width (the distance over which gene-frequencies change)—are particularly commendable. Many of the estimates may be no better than an order of magnitude, but they can nevertheless constrain the biologically feasible solutions to general models in a dramatic and cautionary way. Nevertheless, I was left with the distinct impression that theory can now generate so many alternative explanations for apparently simple phenomena that distinguishing hypotheses would in many cases be a major undertaking, if not downright impossible.

Before I read the book, I knew very little about clines, other than that they were interesting. When I was reading it, there were times when I felt that I was finding out more than I really wanted to know; that somehow, Darwin's clear, simple problem had been lost in the toolkit of modern population genetics. But by the time I finished it, I was more convinced than ever that clines are intriguing. For any biologist brought up in the simple belief that allopatric speciation is all, this book is essential reading.

John H. Lawton

John Lawton is Lecturer in Ecology at the University of York, UK.

African fossil hominids

Catalogue of Fossil Hominids. Part 1: Africa. (Second edition). Edited by K. P. Oakley, B. G. Campbell and T. I. Molleson. Pp.210 + 21 plates. (British Museum Natural History: London 1977.) £12.

REVIEWING a catalogue can be likened to reviewing a telephone directory. It would take a devoted reviewer—nay, an obsessive one—to check all the facts; all one can reasonably do is comment on their presentation.

There are now three published parts of the *Catalogue of Fossil Hominids*. Part I deals with Africa, Part II with Europe, and Part III with the Americas, Asia and Australasia. Part I is the first to go into a second edition. All follow a similar style. Fossils are grouped by site and then, for the large sites, into anatomical subcategories. Eighteen items of information are given about each fossil, or group of fossils, varying from the exact name and location of the site to the location of the moulds for casts. The items include a very brief description of the completeness of the fossils, as well as geological, palaeontological and bibliographic information. The *Catalogue* is clearly intended as a source for reference and information about hominid fossils; how well does the series, and this particular volume, fulfil this role?

As a comprehensive source of information, the series has no rival. My experience is that the volumes represent a very reliable source of data. I detected remarkably few errors in this volume, and the prodigious task of checking entries and proof-reading has been carried out with considerable skill. Once one is accustomed to the layout, information is comparatively easy to retrieve. The use of a bookmark as a key to the various items of information is an excellent idea. (Publishers of Tolstoy, Pasternak and Trollope, please note!)

I would have welcomed clearer information about the circumstances of many of the fossil finds. Were they found 'in situ' or on the surface; and, if on the surface, how reliably can they be associated with particular horizons? Taxonomic attributions are given, but they tend to be hidden away between citations of original reports; perhaps they could be given a section of their own? Inevitably, the choice of which papers to cite is subjective, but I would quibble with some of the selections. It is unfortunate that the paper cited as the first report of KNM-ER 1470 does not mention the specimen in the text at all.

The inclusion of site maps is a welcome introduction, and greater use of geological sections should be encouraged, though it has to be admitted that compound sections can appear misleadingly simple. The decision to continue the use of Nomina Anatomica terms to describe the material is a wise one: even if it does lead to 'ossa facie'

and 'face' being used in the same sentence, it is important to base descriptions on internationally understood terms. The inclusion of photographs of so few specimens, even if they are holotypes, is of questionable value.

I had to work very hard to find even minor points of criticism in the latest volume. Fossils from Sterkfontein are catalogued within Members, but none of the references cited gives information about the definition of the Members; and in the section on Swartkrans reference is made to A4 dating, but this category of dating is not defined in the general introduction; however, these are points of detail and not serious criticisms.

There is no doubt in my mind that the quality of the whole *Catalogue*, and this new volume in particular, is a credit to the editors, and to the scientists who contri-

buted entries from individual countries. The Wenner-Gren Foundation for Anthropological Research is to be congratulated for supporting the preparation of the *Catalogue*. The clear layout and high quality of the presentation do credit to the publishers, who are to be commended for producing such a specialist publication at a realistic price. The *Catalogue* will continue to accumulate entries, and one hopes that the possibility of producing it in a loose leaf format is being explored.

My own copy of the predecessor to this new edition is so well used that it is disintegrating. I am confident the same fate will befall the new edition; I can think of no finer recommendation for a book.

Bernard Wood

Bernard Wood is Senior Lecturer in Anatomy at Middlesex Hospital Medical School, London, UK.

Statistical techniques in biostratigraphy

Concepts and Methods of Biostratigraphy. Edited by Erle G. Kauffman and Joseph E. Hazel. Pp. xiii + 658. (Wiley: New York, London and Toronto, 1977.) £26.25; \$44.45.

So much in geology depends on good biostratigraphy that one must welcome any good new treatment of its concepts, methods and results. This weighty (in more ways than one) volume starts well with a scholarly and absorbing essay by J. M. Hancock on the history of concepts of correlation, and continues with six articles on a variety of biological concepts involving, for example, speciation, dispersal mechanisms and biogeography. There follow a further six articles on biostratigraphic methods, and the remainder of the book deals with a dozen major fossil groups. These include such excellent stratigraphic indices as graptolites, conodonts and ammonites together with a few 'jokers', such as corals and gastropods, which no respectable stratigrapher would use if he could possibly avoid it. Even more surprisingly, there is nothing on the increasingly important nannofossils and dinoflagellates, that have in recent years become extremely valuable in much oil company exploration and deep-sea drilling.

Although the book is marred somewhat by the poor, smudgy quality of many of the illustrations, the editors are to be congratulated on having persuaded so many leading palaeontologists to collaborate in producing a volume that will serve as a most useful teaching aid and reference work. If I

find myself more than a little resistant to the promotional enthusiasm of the blurb writer, it may be because I am not persuaded by what I estimate to be the editors' higher ambitions, judging from their joint preface and individual articles. Hazel wishes to promote the use of a variety of statistical techniques in biostratigraphy, but in my experience good stratigraphic marker fossils are the only crucial consideration. If they are present then statistics are unnecessary and if they are not, no amount of juggling with numbers will be of much help.

Far more interesting in my view is Kauffman's ambition to apply modern evolutionary and ecological concepts to improving correlation as exemplified by his work on the Cretaceous of the US Western Interior. Although I share his fascination with the sorts of biological and geological questions that his work raises, I feel he may perhaps be guilty of putting the cart before the horse. Biostratigraphy is essentially an empirical subject and excellent work can be undertaken by people with little interest in, knowledge of, or talent for biological theory (one has only to think of stratigraphy's prime exemplar, William Smith). Thus, ammonites are more useful in stratigraphy than bivalves because of their much higher rate of faunal turnover, a fact recognised and utilised for well over a century. This raises an intriguing scientific question, as does, says, the existence of faunal provinces, but in both cases attempted solution of the problems is irrelevant to correlation of strata. Good palaeobiology depends on good stratigraphy and not the other way around.

A. Hallam

A. Hallam is Professor of Geology at the University of Birmingham, UK.

obituary

A. R. Luria

ALEXANDER ROMANOVITCH LURIA, the distinguished Soviet neuropsychologist, died in Moscow on 14 August, 1977, at the age of 75.

He was born in 1902 and educated at the Universities of Kazan and Moscow, where he took his degree in psychology, later qualifying also in medicine. A talented experimental psychologist, his best known work was concerned with the analysis of psychological disabilities resulting from war wounds of the brain. But his interests covered a wide range of themes in experimental psychology, in particular problems of language and its acquisition in young children. He paid several visits to this country and a number of his books have been translated into English.

Luria's earliest work was concerned with the analysis of motor disorganisation provoked by conflict and stress. This was based on a long series of experimental studies carried out at the State Institute of Experimental Psychology in Moscow in the 'twenties and published as a book which appeared in English translation in 1932. Although deriving in some measure from Jung and Peterson's work on word-association and the psychogalvanic response, Luria was concerned with the disruption of volitional movement rather than with autonomic changes and did much to extend the use of objective method in the study of emotional stress.

Rather surprisingly, his work owed but little to Pavlov, who at much the same time was investigating experimental neuroses in dogs by conditioned reflex techniques. While Luria held Pavlov's physiological work in the highest esteem, relations between physiology and psychology in the Soviet Union have never been close and few Soviet psychologists espoused behaviourism. As with the great majority of Soviet psychologists, Luria's outlook was far from Pavlovian and his work was wholly confined to the study of human experience and behaviour.

The most important figure in Russian psychology after the Revolution was L. S. Vygotsky, Luria's predecessor in the Chair of Psychology in the University of Moscow and a man who influenced him greatly. Vygotsky's main interests lay in child development, more especially in the study of



language and its relation to intellectual growth. Following Pavlov's suggestion that the main differences between human and animal behaviour are bound up with the evolution of language, Luria concerned himself particularly with the acquisition of language in young children and its effects on the learning of motor skills.

Although relatively little of this work was translated, he brought much of it together in a series of three lectures delivered at University College London in 1958 and subsequently published as a short book under the title of *Speech and the Regulation of Normal and Abnormal Behaviour* (1961). More physiological in tone than Vygotsky, Luria followed him in his full acceptance of the importance of language in the intellectual development of the child. In this respect he stands far closer to Piaget than to Pavlov.

Interested as he was in the acquisition of language, Luria was even more concerned with the nature of its dissolution. His studies of aphasia, somewhat in the tradition of Hughlings Jackson and Henry Head, remain perhaps as his outstanding contribution to neuropsychology. During the last war, Luria was closely concerned with the assessment of psychological disability in brain-injured servicemen and in formulating programmes of rehabilitation based upon thorough psychological examination. His book on *Traumatic Aphasia* (1947, English translation 1970), is especially noteworthy for its detailed analysis of the various patterns of linguistic disability resulting from brain injury and their

relationship to the site and extent of the cerebral lesion.

Some years later, his considered views on the problems of cerebral localisation together with an exposition of his methods for eliciting specific psychological deficits were set out in a noteworthy monograph. This was *Higher Cortical Functions in Man* (1963, English translation 1966), which has had wide influence. Although putting forward no new theory or model of higher nervous activity, the subtlety and intuitive skill displayed by Luria in devising simple methods for analysing cortical defects and evaluating their significance represents a major achievement.

Apart from his scientific preoccupations, Luria was a man with lively human sympathies who achieved an unusual degree of empathy with the victims of brain injury. Among these was the distinguished physicist, L. D. Landau, to whose rehabilitation after a severe head injury Luria devoted quite outstanding skill and care. Nor were his ministrations limited to the famous, as is witnessed by his moving account of the visual and symbolic difficulties of a completely unknown young scientist who had sustained a penetrating occipitoparietal war wound of the dominant cerebral hemisphere. This case is described in *The Man with a Shattered World* (1972), a semi-popular book written with unusual sensitivity and very considerable literary skill. As has been well said elsewhere, Luria was a very warm human being and a deeply compassionate and concerned physician with something of the artist in his composition.

Alexander Luria was a holder of the Order of Lenin and received many other honours from his own country and further afield. He was a foreign member of the National Academy of Sciences of the United States and an Honorary Fellow of the British Psychological Society. He was awarded an Honorary Doctorate of Science by the University of Leicester. He is survived by his widow, herself a medical scientist, and daughter.

O. L. Zangwill

Jean Maetz

JEAN MAETZ died tragically in a road accident in Scotland on 16 August, 1977. He was Chief of the Groupe de Biologie Marine of the Département de

Biologie du Commissariat à L'Energie Atomique located at the Station Zoologique, Villefranche-sur-Mer, France. Born in Mutzig in Alsace in 1922, he was educated at the Ecole Normale Supérieure in Paris, and worked at Saclay before going to Villefranche in 1964.

Early attempts to measure teleostean osmoregulation with radioisotopes had been made in the fifties, and revived by Motais in 1961. Maetz with Motais in 1965 discovered that in sea-water the gill was the major site of sodium exchange, and that up to 100% of the internal sodium could be exchanged each hour. Maetz was quick to realise the importance of techniques which allowed both influx and efflux of ions across the gill to be measured while the salt balance of the animal was not disturbed. He refined and automated these techniques with the effect that a constant stream of papers has flowed from his laboratory in which a variety of perturbations (ionic strength, temperature, hormones) were employed and their effects on branchial fluxes described. He was particularly interested in problems of adaptation in euryhaline fishes and while he stressed the difficulty of obtaining exact thermodynamic data in whole animals he enjoyed building models to describe the data. These were intended as a challenge to others to do experiments designed to test the models.

It is in no small part due to his stimulus that there is a lively interest in branchial transport today. While there is now general agreement about the active transport of chloride in the seawater gill, the mechanism of Na-K exchange is more controversial. Whether exchange proceeds by an active mechanism or is a result of potential changes accompanying alterations in composition of external medium is still a matter of dispute. Although Maetz with Campanini in 1966 were the first to show the change in gill potential which followed when eels were transferred from sea water to fresh water Maetz initially favoured an active mechanism. More recent observations suggest that Na/K exchanges are diffusive in nature, yet awkward observations remain to indicate that some component of the exchange is potential insensitive. It is a tragedy he will not be able to resolve this matter after contributing so much of the information on which the discussion is based.

The impact of the mass of phenomenological data, collected for many different species, which has come from his efforts has perhaps not yet been realised. Its importance to ecology and relevance with regard to the changing environment of the seas and estuaries may be considerable.

More recently he turned to simpler systems such as the perfused gill, work already started with Rankin, and to biochemical and morphological studies of chloride cells. It is to be hoped others will continue to explore his ideas in these areas.

Jean Maetz was a man of great charm with an infectious enthusiasm for research. Many of his former students now hold positions in research institutions and his laboratory was never without visiting scientists, who came from all over the world. A visit to his laboratory was a commitment to do experiments, and often at the end of the day to enjoy the hospitality and friendship offered by his whole family. He is survived by his wife, Betty, and two daughters. *A. W. Cuthbert*

Louis Fieser

LOUIS FREDERICK FIESER was an exemplar of excellence promoted and sustained by Harvard University. Born in Columbus, Ohio, on April 7, 1899, he completed his Ph.D. degree under J. B. Conant at Harvard in 1924. After five years on the staff of Bryn Mawr College (where he discovered his aptitude for teaching) he returned to the Harvard chemistry department in 1930, becoming Sheldon Emery Professor of Organic Chemistry (1939–1968) and Professor Emeritus from 1968 until his death on July 25, 1977.

Among the honours conferred on Fieser were Fellowship of the National Academy of Sciences (1940), and the D.Pharm. *honoris causa* of the University of Paris (1953). From the American Chemical Society he received the Wm. H. Nichols Medal (1963) and the Award in Chemical Education (1967).

Fieser's abounding enthusiasm for investigative organic chemistry was rooted in his own experimental work, which he continued throughout his career. His attachment to bench work is well portrayed in the photograph that accompanied a biographical sketch by Dr Hans Heymann in the *Journal of Organic Chemistry* of June 1965—an issue composed of numerous papers dedicated to Fieser on the occasion of his 66th birthday, by authors from many countries who had been students or post-doctoral workers in the Converse Memorial Laboratory.

Early research by Fieser stimulated developments in several fields which have remained important. In 1939, he established the structure of Vitamin K₁ by a synthesis of characteristic practicality, isolating the natural vitamin from alfalfa extract for comparison. Fieser had earlier devised syntheses of methylcholanthrene and other carcinogenic hydrocarbons, and his varied interests led him to compile (with Mary

Fieser) his books on *Natural Products Related To Phenanthrene* (1936, 1937) in which the developing (and confused) chemistry of the steroids was skilfully elucidated and critically reviewed. In the 1937 volume, Fieser introduced the α/β nomenclature now universally employed to designate hydroxy and other substituent configurations in the steroid nucleus. The 3rd edition appeared in 1949, benefiting from (and contributing to) the burgeoning of steroid research that accompanied the discovery of the therapeutic value of cortisone.

The ensuing decade saw remarkable advances in steroid chemistry and biochemistry which had an impact in much wider fields. Observations by Fieser on the stereochemistry of steroid reactions were among the stimuli that led D. H. R. Barton (while a Visiting Lecturer at Harvard) to develop the principles of conformational analysis. In addition to participating in many aspects of research on steroids, Fieser found time to compile—again with his wife as co-author—the masterly 4th edition of the original book, necessarily retitled *Steroids* (1959) because the wealth of new information compelled the exclusion of other material.

Fieser's insight in selecting significant research topics is exemplified by his early work on benzo[*a*]pyrene, the carcinogenic action of which has only recently been clarified in structural terms. Another of his interests concerned the possibility that carcinogenicity might arise from minor sterols occurring as companions of cholesterol: he isolated a number of such minor components from commercial cholesterol. Although no carcinogenic sterols (with the exception of cholesterol α -oxide) appear to be known, recent work has indicated that certain compounds such as 25-hydroxycholesterol may have significant biochemical effects.

Fieser himself considered that success in teaching was his major accomplishment. At Harvard, his attractive informality as a lecturer won him the students' acceptance of a rigorous introductory course in organic chemistry, generally regarded as 'tough, but well worth it' by the participants—who numbered more than 8,000 during the 30 years of the course. Experimental skill was assiduously inculcated, as in the annual Martius Yellow prize competition for the practical class, in which 'Louie' frequently took part.

Fieser's earliest textbook, *Experiments in Organic Chemistry* (1935), and its painstakingly revised successors, conveyed up-to-date techniques to students world-wide: the colour pictures showing how to construct a hot-plate from a can labelled 'Gorton's Cod Cakes' impressed even those readers

who were unacquainted with those comestibles.

The 1957 edition of *Experiments* included an informative review of some 320 reagents: from this modest chapter, Mary and Louis Fieser were to develop their monumental series of handbooks entitled *Reagents for Organic Synthesis*—five volumes comprising more than 4,000 pages of invaluable information and references for research workers.

During the Second World War, Fieser's attention was diverted to investigations on antimalarial drugs of naphthoquinone type. He also played a leading role in the development of incendiary preparations, including napalm. It is clear from his personal account of this work, in the book *The Scientific Method* that while Fieser relished this challenge to the skill of his research team, he was impatient to resume his own research at the earliest opportunity.

Meanwhile, his enforced travels on war service provided some spare hours in which he began (in collaboration with Mary) the first of a series of sparkling and scholarly textbooks on organic chemistry, in which the excitement of discovery was conveyed in a lively, lucid and gracefully literate style. These works deservedly achieved great international success, both in the original and in translations. The first Japanese edition of the *Textbook of Organic Chemistry* appeared in 1952 just as peace was formally resumed between the U.S.A. and Japan: its frontispiece portrayed the Fiesers' Siamese cat accompanied by one of Japanese breed, symbolising the authors' hope that their work would promote reconciliation.

In later years, the Fiesers produced two further books for advanced students, *Advanced Organic Chemistry* (1961) and *Topics in Organic Chemistry* (1963) together with a *Style Guide for Chemists* and other works. Fieser also promoted the study of stereochemistry by means of his inexpensive molecular models based on those of Dreiding.

It was typical of Fieser's determination that after undergoing surgery for a lung tumour he became a campaigner on the risks of smoking, in addition to pursuing his customary literary work. The elegance of the latter matched that of his Siamese cats, but in his perseverance Fieser was more appropriately described by his mottoes, which were *Omnia possum* and *Labor omnia vincit*.

The human warmth implicit in Fieser's books prompted affection for the author in many readers who had no opportunity of direct contact with him. Friends and disciples known and unknown will salute the memory of a

creative and courageous chemist, and offer their condolences to Mary, his wife and scientific partner throughout his career.

C. J. W. Brooks

Sir Frederic Williams

PROFESSOR Sir Frederic Williams, FRS, who died after a year-long illness on 11 August 1977 had held the Edward Stocks Massey Chair of Electro-Technics at the University of Manchester since 1946; he was famed for his pioneer work on electronic circuitry and digital computers, and was perhaps less well known for his variable-speed induction motors and his new transmission system for a motor-car.

Frederic Calland Williams was born on 26 June 1911. He was educated at Stockport Grammar School, the University of Manchester (B.Sc. and Fairbairn Prize 1932, M.Sc. 1933, D.Sc. 1939) and Magdalen College, Oxford (D.Phil. 1936). He joined the staff of the Electro-Technics Department at Manchester in 1936, but left in 1939 to join the Scientific Civil Service at Bawdsey. During the war he worked on radar at TRE (Telecommunications Research Establishment; now the Royal Radar Establishment). It was at TRE that his inherent ability in circuitry became apparent. Those were the days of valves and he used them in unconventional ways to generate the waveforms required in the rapidly developing field of radar. There were circuits actively using all the electrodes of a pentode; diodes were employed for 'catching'; feedback (both positive and negative) was used. It is believed that Williams introduced the 'virtual earth' idea which so simplifies the analysis of negative feedback amplifiers. At TRE he also made contributions to servo-mechanisms.

When he became Professor at Manchester, all the war-time experience was incorporated in new courses which he gave to undergraduates (the whole staff attending, too). This experience also enabled him to start new research lines in his department. Most significantly, he brought to Manchester an idea for a digit store for a computer. There were computers in 1946, but they lacked storage. The Williams device stored binary digits as a charge pattern on the screen of a cathode ray tube. The pattern was scanned and read and continuously 'refreshed' by re-writing from the read-out. It was possible to inspect any point of the pattern at random; access time was thus shorter than in acoustic delay lines—a rival system of storage being developed elsewhere.

A complete prototype computing machine incorporating the Williams tube store was built in the laboratory

at Manchester and a commercial version was produced by Ferranti Ltd., who installed about 20 computers in various establishments in this country and abroad. Early in the development of the second Manchester machine, Professor Williams' interest in computers waned and he took up other interests. However, he must surely be of that small company of men deserving the title of 'Father of the Computer.'

The new interest was induction machinery. The induction motor is essentially a constant-speed machine, although there are machines with two separate windings either of which can be excited to give a two-speed machine. Williams sought to make an induction motor whose speed could be continuously variable over a range and all of whose windings were used all the time. There was the disc motor derived from the linear induction motor; this was a flea-power machine which demonstrated the practicability of the idea, but it led to the spherical motor in which almost the whole surface of the rotor was in active use. A large motor of this type was made by Metropolitan-Vickers Electrical Company, but the machining of spherical surfaces proved difficult and this may be why no further machines were made.

There followed the 'log-motor' (a machine of amazing ingenuity), the phase-change motor (in which two-thirds of the windings were supplied through variable phase changers), several machines with discrete speeds and an induction-excited alternator. He also produced slow high-torque motors using the direct pull between magnetised surfaces.

Whatever his interest at a particular time, Professor Williams spent practically all his time in the laboratory or the workshop. Administrative work was left as much as possible to others and the time spent at his desk was minimised. He was nearly always to be found, in a cloud of tobacco smoke, supervising the construction of a device or its testing.

He was awarded honorary doctorates from four Universities and was elected to Fellowship of the Royal Society in 1950. He received many medals and other awards. He was made a Knight Bachelor in the Birthday Honours in 1976. In his own University he was held in great respect and affection and he ran his Department with an easy authority; his staff pursued their own interests and under his guidance their individual abilities developed. More than 20 of his protégés are now Professors.

Sir Frederic is survived by Lady Williams, a son, a daughter and four grandsons.

L. S. Piggott

Reports and Publications

UK and Ireland—September

- Moon, Mars and Meteorites. Pp. 36. (London: HMSO, 1977. Published for the Institute of Geological Sciences.) 70p net. [310]
- International Planned Parenthood Federation. Occasional Essay No. 5: Health, Nutrition and Population in Human Settlements. By Fred T. Sai. Pp. 32. (London: International Planned Parenthood Federation, 18-20 Lower Regent Street, SW1, 1977.) 85p; \$1.45. [310]
- Health and Safety Commission. Report 1974-76. Pp. 51. (London: HMSO, 1977.) £2 plus postage. [310]
- National Institute of Agricultural Botany. Fifty-Seventh Report and Accounts, 1976. Pp. 110. (Cambridge: National Institute of Agricultural Botany, 1977.) [310]
- Office of Population Censuses and Surveys. Mortality Statistics: Accidents and Violence. (Review of the Registrar General on Deaths Attributed to Accidental and Violent Causes in England and Wales, 1975.) (Series DH4 No. 2). Pp. viii+37. (London: HMSO, 1977.) £1.25 net. [310]
- Thames Water Statistics—1976. Vol. 2: Water Quality Statistics, 1974/75/76. (London: Thames Water Authority, New River Head, Rosebery Avenue, EC1, 1977.) [310]
- University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1976-1977. (Supplement (3) to *Oxford University Gazette*, No. 3707.) Pp. 39-61. (Oxford: The University, 1977.) 20p. [310]
- Oystercatchers and Shellfish: Predator/Prey Studies. By John Goss-Custard, Selwyn McGrorty and Chris Reading. Pp. 10. (Cambridge: Institute of Terrestrial Ecology, Natural Environment Research Council, 1977.) 60p net. [410]
- Memoirs of the Royal Astronomical Society. Vol. 84, Part 2: The Luminosity Distribution of Globular Clusters in the Virgo Cluster of Galaxies. By David A. Hanes. Observations of 104 Extragalactic Radio Sources with the Cambridge 5-km Telescope at 5 GHz. By C. J. Jenkins, G. G. Pooley and J. M. Riley. A Search for Beta Canis Majoris Stars. By L. A. Balona. Radial Velocities of Southern B Stars Determined at the Radcliffe Observatory—VIII. Stars with HD Spectral Types B8 and B9. By Roger Wood. Pp. 45-134. (Oxford and London: Blackwell Scientific Publications, 1977. Published for the Royal Astronomical Society.) [510]
- Green Crop Fractionation. (Proceedings of a Symposium organised by the British Grassland Society and the British Society of Animal Production, 25-26 November, 1976, held at Harrogate, Yorkshire.) Edited by R. J. Wilkins. Pp. vii+189. (Occasional Symposium No. 9.) (Hurley, Maidenhead: British Grassland Society, 1977.) £5. [610]
- Department of Health and Social Security. Nutrition Education: Report of a Working Party. (British Nutrition Foundation. Department of Health and Social Security. Health Education Council.) Pp. v+30. (London: Department of Health and Social Security, 1977. Obtainable from HMSO.) [610]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1342: The Interaction of Large Amplitude Barotropic Waves with an Ambient Shear Flow: Critical Flows. By E. Varley, J. Y. Kazakia and P. A. Blythe. Pp. 189-236. UK £2.90; Overseas £3. Vol. 287, No. 1343: Semi-Classical Mechanics in Phase Space: a Study of Wigner's Function. By M. V. Berry. Pp. 237-271. UK £2.10; Overseas £2.20. (London: The Royal Society, 1977.) [610]
- Meteorological Office. Geophysical Memoirs No. 120: Average Temperatures, Contour Heights and Winds at 30 Millibars Over the Northern Hemisphere. By R. A. Ebdon. Pp. v+170 (48 plates). (London: HMSO, 1977.) £11.50 net. [710]
- Annual Report of the Soil Survey, England and Wales, 1976. Pp. 43. (Harpenden, Herts: The Soil Survey, Rothamsted Experimental Station, 1977.) [1010]
- Royal Observatory Annals. No. 11: Photoheliographic Results 1967. Pp. 114. (Herstmonceux Castle, Hailsham, Sussex: Royal Greenwich Observatory, 1975.) £2.65 net. [1010]
- Progress in Crystal Growth and Characterization*, Vol. 1, No. 1, 1977. Edited by Dr. Brian R. Pamplin. Pp. 1-91. Published as one volume of four parts per year. 1977 Subscription rate: \$45. (Oxford: Pergamon Press, Ltd., 1977.) [1110]
- Medical Research Council. Senile and Presenile Dementias: a Report of the MRC Subcommittee. Compiled by W. A. Lishman. Pp. iv+23. (London: MRC, 1977.) [1310]
- Rheumatology Workshop: a Modern Review of Geigy Pyrazoles. (Proceedings of a Meeting held at Grand Hotel Verdala, Rabat, Malta, G. C., 9th-13th March 1977.) (*The Journal of International Medical Research*, Vol. 5, Supplement 2.) Pp. 120. Symposium on Danol (Danazol), held at The Royal College of Physicians, London, England, on 29th April 1977. (*The Journal of International Medical Research*, Vol. 5, Supplement 3.) Pp. 127. (Northampton: Cambridge Medical Publications, Ltd., 1977.) [1310]
- Department of the Environment. Welsh Office. Development Control Statistics 1975-76. Pp. v+44. (London: Department of the Environment, 20 Albert Embankment, 1977.) £1. [1310]
- Progress in Nuclear Energy*, Vol. 1, No. 1, New Series. Executive Editors: M. M. R. Williams and R. Sher. Pp. 1-71. (Oxford and New York: Pergamon Press, 1977.) £10.50. [1310]
- Radiating Cosmic Dust. By C. D. Andriess. Pp. 107-190. (*Vistas in Astronomy*, Vol. 21, Part 2.) (Oxford and New York: Pergamon Press, 1977.) £8.30. [1310]

- Withdrawals of Authorisations for the use of NHS Hospital Accommodation and Services by Private Patients. (Proposals made by the Health Services Board under Section 4 of the Health Services Act 1976.) Pp. 23. (London: HMSO, 1977.) 45p net. [1410]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1344: The Instability of the Thin Vortex Ring of Constant Vorticity. By Sheila E. Widnall and Chon-Yin Tsai. Pp. 273-305 + plate 1. (London: The Royal Society, 1977.) UK £2.15; Overseas £2.25. [1710]
- Welsh Plant Breeding Station. Annual Report for 1976. Pp. 216. (Plas Gogerddan, Near Aberystwyth: Welsh Plant Breeding Station, University College of Wales, 1977.) £2. [1910]
- Native Pinewood of Scotland: Proceedings of Aviemore Symposium, 1975. Edited by Dr. R. G. H. Bunce and Mr. J. N. R. Jeffers. Pp. x+120. Cambridge: Natural Environment Research Council, Institute of Terrestrial Ecology, 68 Hills Road, 1977.) £2.50 net. [2010]
- Outlook*, No. 1, Autumn 1976. (Published Bi-Annually for Open University graduates.) Pp. 1-16. (Walton Hall, Milton Keynes: The Open University, 1977.) [2010]
- Proceedings of the Royal Society of London. B: Biological Sciences. Vol. 199, No. 1134: A Discussion on Technologies for Rural Health. Organised by D. A. J. Tyrrell, F. R. S., D. P. Burkitt, F. R. S., and Sir William Henderson, F. R. S. Pp. 1-187. (London: The Royal Society, 1977.) UK £5.10; Overseas £5.30. [2010]
- National Institute for Medical Research. Report for 1976-77. Pp. iii+122. (London: Medical Research Council, 1977.) [2010]
- University of London. University College Calendar 1977-78. Pp. 186. (London: University College, 1977.) [2110]
- Department of Health and Social Security. Smoking and Professional People. Pp. 10. (London: Department of Health and Social Security, 1977. Available from HMSO.) [2410]
- Department of Industry. Changes in the Population of Persons with Qualifications in Engineering, Technology and Science, 1959 to 1976. (Studies in Technological Manpower, No. 6.) Pp. viii+51. (London: HMSO, 1977.) £3.25 net. [2410]
- International Union of Pure and Applied Chemistry. Physicochemical Measurements: Catalogue of Reference Materials from National Laboratories. Pp. 505-515. (Oxford and New York: Pergamon Press, 1977.) £3.30. [2610]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 280, No. 975: Pleistocene History of the Vale of St. Albans. By P. L. Gibbard. Pp. 445-483. UK £3.25; Overseas £3.35. Vol. 280, No. 976: The Structure of Plankton Communities. By J. H. Steele and B. W. Frost. Pp. 485-534 UK £3.50; Overseas £3.60. Vol. 280, No. 977: Some New Namurian Bivalve Faunas and Their Significance in the Origin of *Carbonicola* and in the Colonization of Carboniferous Deltaic Environments. By R. M. C. Eager. Pp. 535-570 + plates 1-4. UK £2.95; Overseas £3.05. (London: The Royal Society, 1977.) [2610]
- Snowdon. Pp. 7. (Cheltenham: Countryside Commission, John Dower House, Crescent Place, 1977.) *gratis*. [3110]
- Centre National pour l'Exploitation des Océans. Rapport Annuel 1976. Pp. 87. (Paris: Centre National pour l'Exploitation des Océans, 1977.) [310]
- Institut National de la Recherche Agronomique. Service d'Étude des Sols et de la Carte Pédologique de France. Carte Pédologique de France à 1:100,000—Dijon. Notice Explicative par J. Chretien. Pp. 218 (Versailles: Centre National de Recherches Agronomiques, 1976.) F.53.50. [310]
- Republic of South Africa: Department of Industries. Sea Fisheries Branch Investigational Report No. 113: Stock Differentiation and Growth of the Southern African Kinglip *Genypterus capensis*. By A. L. L. Payne. Pp. 32. (Sea Point, Cape Town: Sea Fisheries Branch, Department of Industries, 1977.) [510]
- United States Department of the Interior: Geological Survey. Professional Paper. 994-A: Depositional Environment of Upper Cretaceous Black Sandstones of the Western Interior. By Robert S. Houston and John F. Murphy. Pp. v+29. (Washington, DC: US Government Printing Office, 1977.) [510]
- Siemens Forschungs- und Entwicklungsberichte/Research and Development Reports, 5/77, Bd. 6. Edited by Hans Suchlandt. Pp. 263-321. (Berlin and New York: Springer-Verlag, 1977.) [610]
- United States Department of the Interior: Geological Survey. Bulletin 1278-E: Geochemical Exploration Techniques Based on Distribution of Selected Elements in Rocks, Soils, and Plants, Vekol Porphyry Copper Deposit Area, Pinal County, Arizona. By Maurice A. Chaffee. Pp. v+78. (Washington, DC: US Government Printing Office, 1977.) [610]
- Indian Council of Medical Research. Annual Report of the Director-General, 1976. Pp. 168. (New Delhi: Indian Council of Medical Research, 1977.) [610]
- Swedish College of Forestry. Studia Forestalia Suecica. Nr. 137: Observations on Trees of Scots Pine (*Pinus sylvestris* L.) and Lichens Around a HF and SO₂ Emission Source. By Jan-Erik Hällgren and Bengt Nyman. Pp. 40. Nr. 138: Site Index Estimation by Means of Site Properties, Scots Pine and Norway Spruce in Sweden. By Björn Hägglund and Jan-Erik Lundmark. Pp. 38. Nr. 139: Isozyme Studies in Seed Orchards. By Dag Rudin and Dag Lindgren. Pp. 23. (Stockholm: Swedish College of Forestry, 1977. Obtainable from Liber Distribution, S-162 89 Vällingby, Sweden.) [610]
- CERN—European Organization for Nuclear Research. CERN 77-16: K⁻ and K⁻p Elastic Scattering in K⁻d Collisions from 1.2 to 2.2 GeV/c. By Y. Decais, J. Duchon, M. Louvel, J.-P. Patry, J. Seguinot, P. Baillon, C. Bricman, M. Ferro-Luzzi, J.-M. Perreau and

- T. Ypsilantis. Pp. vi+86. (Geneva: CERN, 1977.) [710]
- Maribana Research Findings: 1976. Edited by Robert C. Petersen. (NIDA Research Monograph 14.) (US Department of Health, Education and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration.) Pp. vi+251. (Washington, DC: US Government Printing Office, 1977.) [1010]
- Department of Health, New Zealand. Environmental Radioactivity—Annual Report 1976. Pp. 13. (Christchurch: National Radiation Laboratory, 1977.) [1010]
- Smithsonian Contributions to Zoology, No. 239: Comparative Ethology of the Large-spotted Genet (*Genetta tigrina*) and Some Related Viverrids. By Christen M. Wemmer. Pp. iii+93. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [1010]
- National Institute on Drug Abuse. Research Monograph Series, No. 13: Cocaine—1977. Edited by Robert C. Petersen and Richard C. Stillman. Pp. 223. (Rockville, Md.: Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, 1977. For sale by US Government Printing Office, Washington, DC.) \$3. [1310]
- Fluid Phase Equilibria*, Vol. 1, No. 1, September 1977. Pp. 1-91. Subscription for 1977/78: \$58.95; Dfl. 144. Free sample copies available. (Amsterdam: Elsevier Scientific Publishing Company, 1977.) [1310]
- Intelligence*, Vol. 1, No. 1, January 1977. Edited by Douglas Detterman. Pp. 1-125. Published quarterly. Subscription Vol. 1, 1977: \$38; Personal Subscription \$15. (Norwood New Jersey: Ablex Publishing Corporation, 1977.) [1410]
- Cognitive Science*, Vol. 1, No. 1, January 1977. Edited by Roger Schank, Allan Collins and Eugene Charniak. Pp. 1-123. Published quarterly. Subscription, Vol. 1, 1977: \$38; Personal subscription \$15. (Norwood, New Jersey: Ablex Publishing Corporation, 1977.) [1410]
- Smithsonian Contributions to Zoology, No. 252: *Anisochromia straussi*, New Species of Protogynous Hermaphroditic Fish, and Synonymy of *Anisochromidae*, *Pseudopleurostomidae*, and *Pseudochromidae*. By Victor G. Springer, C. Lavett Smith, and Thomas H. Fraser. Pp. 15. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [1410]
- A Report on the Lawrence Berkeley Laboratory. Pp. 67. (Berkeley, Calif.: Lawrence Berkeley Laboratory, University of California, 1977.) [1410]
- International Development Research Centre, Ottawa. On Common Ground: Report on the activities of IDRC 1976/1977. Pp. 30. (Ottawa: IDRC, 1977.) [1710]
- United States Department of the Interior: Geological Survey. Professional Paper 1020: Ordovician and Silurian Graptolite Succession in the Trail Creek Area, Central Idaho—a Graptolite Zone Reference Section. By Claire Carter and Michael Churkin. Jr. Pp. iv+36 + plates 1-7. (Washington, DC: US Government Printing Office, 1977.) [1710]
- CERN: European Organization for Nuclear Research. CERN 77-15: ISR Performance for Pedestrians. By K. Hübner. Pp. 55. (Geneva: CERN, 1977.) [1710]
- Australian Institute of Marine Science. Monograph Series, Vol. 1: Scleractinia of Eastern Australia. Part I: Families Thamnasteriidae, Astrocoeniidae, Pocilloporidae. By J. E. N. Veron and Michel Pichon. Pp. 86. (Townsville, Qd.: Australian Institute of Marine Science, 1976.) A\$4.70 plus postage. [1810]
- The Study of the Future: An Agenda for Research. Edited by Wayne I. Boucher. With contributions by Roy Amara *et al.* Pp. ix+316. (Washington, DC: National Science Foundation, 1977. Obtainable from US Government Printing Office, Washington.) \$4.75. [2010]
- The Pasteur Institute of Southern India, Coonoor. Annual Report of the Director 1975, and Scientific Report 1976. Pp. 68. (Coonoor, S. India: The Pasteur Institute of Southern India, 1977.) [2010]
- Smithsonian Contributions to Paleobiology, No. 33: Evolution of *Oblitacanth* from *Paleocosta* (Ostracoda: Trachyleberididae) During the Cenozoic in the Mediterranean and Atlantic. By Richard H. Benson. Pp. iii+47. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [2010]
- Government of India. Annual Report of the Department of Space, 1976/1977. Pp. 64. (Bangalore: Department of Space, Government of India, 1977.) [2110]
- Etude de l'Accélération de la Diffusion dans l'Or et dans l'Aluminium sous Irradiation Neutronique. Par Denis Acker. (Thèse présentée à l'Université de Paris Sud pour obtenir le Grade de Docteur en Sciences.) Pp. 154. (C. E. N. de Saclay, B. P. No. 2, 91190 Gif-sur-Yvette; Commissariat à l'Énergie Atomique, Division de Métallurgie et d'Étude des Combustibles Nucleaires, 1977.) [2110]
- National Science Council, Taipei, Taiwan. NSC Review, 1975-76. Pp. 136. (Taipei, Taiwan: National Science Council, Republic of China, 1977.) [2410]
- World Health Organization. Technical Report Series, No. 611: Use of Ionizing Radiation and Radionuclides on Human Beings for Medical Research, Training, and Nonmedical Purposes—Report of a WHO Expert Committee. Pp. 39. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 6; \$2.40. [2410]
- Australia: Commonwealth Scientific and Industrial Research Organization. CSIRO Plant Industry 1976. Pp. 146. (East Melbourne: CSIRO, 1977.) [2410]
- Australian Journal of Zoology*, Supplementary Series, No. 50: The Generic Classification of the Bolboceratini of the Australian Region, with Descriptions of Four New Genera (Scarabaeidae: Geotrupinae). By H. F. Howden and J. B. Cooper. Pp. 50. No. 51: A Revision of Australian Fanniidae (Diptera: Calyptrata). By A. C. Pont. Pp. 60. (East Melbourne: Editorial and Publications Service, CSIRO, P.O. Box 89, 1977.) [2410]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Animal Health, 1976. Pp. 160. (East Melbourne: CSIRO, 1977.) [2410]

Recent scientific and technical books

Mathematics

- ALTMAN, M. *Contractors and Contractor Directions: Theory and Applications—a New Approach to Solving Equations.* (Lecture Notes in Pure and Applied Mathematics, Vol. 32.) Pp.x+290. ISBN-0-8247-6672-5. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr80.
- BAILEY, Jr., W. L., and SHIODA, T. (edited by). *Complex Analysis and Algebraic Geometry.* (A Collection of Papers Dedicated to K. Kodaira.) Pp.xii+401. ISBN-0-521-21777-6. (Cambridge, London and New York: Cambridge University Press, 1977.) £28.
- BERGER, Melvin S. *Nonlinearity and Functional Analysis: Lectures on Nonlinear Problems in Mathematical Analysis.* (Pure and Applied Mathematics: A Series of Monographs and Textbooks.) Pp. xix+417. ISBN-0-12-090350-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$24.50; £17.40.
- BIGGS, Norman. *Interaction Models.* (Course given at Royal Holloway College, University of London, October–December 1976. London Mathematical Society Lecture Note Series, No.30.) Pp.101. ISBN-0-521-21770-9. (Cambridge, London and New York: Cambridge University Press, 1977.) £4.50.
- CHADWICK, P. *Continuum Mechanics: Concise Theory and Problems.* Pp.174. ISBN-0-04-510057-8. (London: George Allen and Unwin, Ltd., 1976.) £3.95.
- DIEUDONNÉ, J. *Treatise on Analysis, Vol. 5.* Translated by I. G. Macdonald. (Pure and Applied Mathematics: A Series of Monographs and Textbooks, Vol. 10-V.) Pp.xii+243. ISBN-0-12-215505-X(v.5). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$21.50; £15.25.
- FIENBERG, Stephen E. *The Analysis of Cross-Classified Categorical Data.* Pp.x+151. ISBN-0-262-06063-9. (Cambridge, Mass. and London: the MIT Press, 1977.) \$10.95.
- GIBBONS, Jean Dickinson, OLKIN, Ingram, and SOBEL, Milton. *Selecting and Ordering Populations: A New Statistical Methodology.* (A Wiley Publication in Applied Statistics.) Pp.xxi+569. ISBN-0-471-02670-0. (New York and London: John Wiley and Sons, 1977.) \$31.70; £18.75.
- GONZALEZ, Rafael, and WINTZ, Paul. *Digital Image Processing.* (Applied Mathematics and Computation, No.13.) Pp.xvi+431. ISBN-0-201-02597-3. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1977.) Hard binding \$29.50; Paper binding \$19.50.
- GREEN, J. R., and MARGERISON, D. *Statistical Treatment of Experimental Data.* Pp.x+382. ISBN-0-444-41615-3. Amsterdam: Elsevier Scientific Publishing Company, 1977. Distributed in the USA and Canada by Elsevier North-Holland, Inc., New York.) Dfl. 85; \$34.95.
- IYANAGA, S., and KAWADA, Y. (edited by). *Encyclopedic Dictionary of Mathematics.* By the Mathematical Society of Japan. Translation reviewed by Kenneth O. May. Vol. 1: xiv+1-883. Vol. 2: Pp.885-1750. ISBN-0-262-09016-3. (Cambridge, Mass. and London: The MIT Press, 1977.) £80.
- MASON, J. David (edited by). *Proceedings of the Conference on Stochastic Differential Equations and Applications.* Park City, Utah, February 17-20, 1976. Pp.ix+253. ISBN-0-12-478050-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$10.50; £7.45.
- MICCHELLI, Charles A., and RIVLIN, Theodore J. (edited by). *Optimal Estimation in Approximation Theory.* (The IBM Research Symposia Series.) Pp.ix+300. ISBN-0-306-31049-X. (New York and London: Plenum Press, 1977.) \$35.40.
- RABINOWITZ, Paul H. (edited by). *Applications of Bifurcation Theory.* (Proceedings of an Advanced Seminar Conducted by the Mathematics Research Center, The University of Wisconsin at Madison, October 27-29, 1976. Publication No. 38.) Pp.ix+389. ISBN-0-12-574250-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$15.50; £11.
- SZMYDT, Zofia. *Fourier Transformation and Linear Differential Equations.* Translated from the Polish by Marcin E. Kuczmarski. Pp.xix+503. ISBN-0-277-0622-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, Warszawa: PWN—Polish Scientific Publishers, 1977.) Dfl. 90; \$34.
- VAN RYZIN, J. (edited by). *Classification and Clustering.* (Proceedings of an Advanced Seminar Conducted by the Mathematics Research Center, The University of Wisconsin at Madison, May 3-5, 1976.) Pp.x+467. ISBN-0-12-714250-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17; £12.05.

Astronomy

- COLLINS, Mike (compiled by). *Astronomical Catalogues 1951-1975.* (Bibliography Series No.2.) Pp.ix+325. ISBN-0-85296-440-4. (Hitchin, Herts.: INSPEC, The Institution of Electrical Engineers, 1977.) UK £70; rest of the world £80.
- DE JONG, T., and MAEDER, A. (edited by). *Star Formation.* (International Astronomical Union/Union Astronomique Internationale. Symposium No. 75, held in Geneva, Switzerland, September 6-10, 1976.) Pp.xiv+296. ISBN-90-277-0796-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Cloth Dfl. 75; \$30. Paper Dfl. 45; \$18.
- JAUNCEY, David L. (edited by). *Radio Astronomy and Cosmology.* (International Astronomical Union/Union Astronomique Internationale. Symposium No. 74, held at Cavendish Laboratory, Cambridge, England, August 16-20, 1976.) Pp.xx+398. ISBN-90-277-0838-X. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Cloth Dfl. 95; \$38. Paper Dfl. 50; \$19.50.
- MITTON, Simon (editor-in-chief). *The Cambridge Encyclopaedia of Astronomy.* Pp.481. ISBN-0-224-01418-8. (London: Jonathan Cape, Ltd., 1977.) £15 net.
- MÜLLER, Edith (edited by). *Highlights of Astronomy, Vol. 4.* (As presented at the 16th General Assembly, 1976. International Astronomical Union/Union Astronomique Internationale.) Part 1: Pp.370. ISBN-90-277-0849-5. Cloth Dfl. 85; \$34. Paper Dfl. 55; \$22. Part 2: Pp.403. ISBN-90-277-0850-9. Cloth Dfl. 95; \$38. Paper Dfl. 62.50; \$25. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.)
- MÜLLER, Edith A., and JAPPEL, Arnost (edited by). *Proceedings of the Sixteenth General Assembly, Grenoble 1976.* (International Astronomical Union/Union Astronomique Internationale. Transactions of the International Astronomical Union, Vol. 16B.) Pp.x+586. ISBN-90-277-0836-3. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 125; \$50.
- ROWAN-ROBINSON, Michael. *Cosmology.* (Oxford Physics Series.) Pp.x+158. ISBN-0-19-851838-2. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) Boards £6 net; Paper £2.95 net.

Physics

- BRAGINSKY, V. B., and MANUKIN, A. B. *Measurement of Weak Forces in Physics Experiments.* Edited by David H. Douglass. Pp.xiii+153. ISBN-0-226-07070-0. (Chicago and London: The University of Chicago Press, 1977.) £7.
- CHEN, Chih-Wen. *Magnetism and Metallurgy of Soft Magnetic Materials.* (Series of Monographs on Selected Topics in Solid State Physics.) Pp.xviii+571. ISBN-0-7204-0706-0. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 175; \$71.50.
- CHOQUET-BRUHAT, Yvonne, DEWITT-MORETTE, Cécile, and DILLARD-BLEICK, Margaret. *Analysis, Manifolds and Physics.* Pp.xvii+544. ISBN-0-7204-0494-0. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 49.50; \$19.50.
- DUGDALE, J. S. *The Electrical Properties of Metals and Alloys.* (The Structures and Properties of Solids, 5.) Pp.292. ISBN-0-7131-2524-1. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £10.50; Paper £4.95.
- FLUENDY, M. A. D. *Chemical Applications of Molecular Beam Scattering.* (Studies in Chemical Physics.) Pp.xi+400. ISBN-0-412-15550-8. (London: Chapman and Hall, 1977.) Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £5.50.
- HENDERSON, B., and WERTZ, J. E. *Defects in the Alkaline Earth Oxides, with Applications to Radiation Damage and Catalysis.* (Taylor and Francis Monographs on Physics.) Pp.159. ISBN-0-85066-086-6. (London: Taylor and Francis, Ltd., 1977.) £8.50 net.
- KALDIS, E., and SCHEEL, H. J. (edited by). *1976 Crystal Growth and Materials.* (A Selection

- of Review Papers Presented at the First European Conference on Crystal Growth ECCG-I and Materials Symposium, Zurich, Switzerland, 12-18 September 1976.) (Current Topics in Materials Science, Vol. 2.) Pp.xvi+916. ISBN-0-7204-0740-0. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 300; \$122.50.
- KANTOR, Frederick W. *Information Mechanics.* Pp.xiii+397. ISBN-0-471-02968-8. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$27.90; £16.45.
- KNOEPFEL, H. (edited by). *Tokamak Reactors for Breakeven: a Critical Study of the Near-Term Fusion Reactor Program.* Erice-Trapani (Sicily), September 21-October 1, 1976. (International School of Fusion Reactor Technology, "Ettore Majorana" Centre for Scientific Culture.) Pp.659. ISBN-0-08-022034-7. (Oxford and New York: Pergamon Press, 1978. Published for the Commission of the European Communities.) £30.50.
- LEVY, R. A., and HASEGAWA, R. (edited by). *Amorphous Magnetism II.* Pp.xii+680. ISBN-0-306-34412-2. (New York and London: Plenum Press, 1977.) \$71.40.
- LIETH, R. M. A. (edited by). *Preparation and Crystal Growth of Materials with Layered Structures.* (Physics and Chemistry of Materials with Layered Structures, Vol. 1.) Pp.viii+280. ISBN-90-277-0638-7. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 95; \$38.
- McCAIG, Malcolm. *Permanent Magnets in Theory and Practice.* Pp.374. ISBN-0-7273-1604-4. (London and Plymouth: Pentech Press, 1977.) £12.50.
- SAXE, Robert L. *The Chain Photon Theory of Matter and Energy.* Pp.xxii+340. (New York: Robert L. Saxe, 1977.) np.
- SHARP, Robert T., and KOLMA, Bernard (edited by). *Group Theoretical Methods in Physics.* (Proceedings of the Fifth International Colloquium, Université de Montréal, July 1976.) Pp.xvi+668. ISBN-0-12-637650-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$29.50; £20.95.
- SPEAR, W. E. (edited by). *Amorphous and Liquid Semi-conductors.* (Proceedings of the Seventh International Conference, Edinburgh, June 27-July 1, 1977.) Pp.xv+891. (Edinburgh: Centre for Industrial Consultancy and Liaison, University of Edinburgh, 1977. Published on behalf of the Conference Committee.) £37.50.

Chemistry

- ANDEREGG, G. (prepared for publication by). *Critical Survey of Stability Constants of EDTA Complexes.* (International Union of Pure and Applied Chemistry, Analytical Chemistry Division, IUPAC Chemical Data Series, No. 14.) Pp.42. ISBN-0-08-022009-6. (Oxford and New York: Pergamon Press, 1977.) £3.90.
- BARNES, A. J., ORVILLE-THOMAS, W. J. (edited by). *Vibrational Spectroscopy—Modern Trends.* Pp.xiii+442. ISBN-0-444-41632-3. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 122; \$49.95.
- BERNE, Brice J. (edited by). *Statistical Mechanics. Part B: Time-Dependent Processes.* (Modern Theoretical Chemistry, Vol. 6.) Pp.xv+362. ISBN-0-306-33506-9(v.6). (New York and London: Plenum Press, 1977.) \$47.40.
- BRAME, Jr., Edward F., and GRASELLI, Jeanette G. (edited by). *Infrared and Raman Spectroscopy.* Part C. Pp.ix+717-1039. ISBN-0-8247-6527-3. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr8.118.
- BULLOCK, J. D. (senior reporter). *Biosynthesis, Vol. 5. (A Review of the Literature Published during 1975 and 1976.)* (A Specialist Periodical Report.) Pp.ix+318. ISBN-0-85186-543-7. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Leichworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £22.50; \$45.
- CAMPBELL, Ian M. *Energy and The Atmosphere: a Physical-Chemical Approach.* Pp.ix+398. ISBN-0-471-99482-0. (London and New York: John Wiley and Sons, Ltd., 1977.) Cloth £14.50; \$31. Paper £5.95; \$12.50.
- CRABBE, Pierre (edited by). *Prostaglandin Research.* (Organic Chemistry: A Series of Monographs, Vol. 36.) Pp.xv+341. ISBN-0-12-194660-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$26; £18.45.
- CROMPTON, T. R. *Chemical Analysis of Additives in Plastics.* Second edition. (International Series in Analytical Chemistry, Vol. 46.) Pp.xii+366. ISBN-0-08-020497-X. (Oxford and New York: Pergamon Press, 1977.) £15.25.
- DAY, P. (senior reporter). *Electronic Structure and Magnetism of Inorganic Compounds, Vol. 5. (A Review of the Literature Published during 1974 and 1975.)* (A Specialist Periodical Report.) Pp.viii+248. ISBN-0-85186-291-8. (London: The Chemical Society, 1977. Obtainable from Chemical Society, Blackhorse Road, Leichworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £21; \$42.
- GUYOT, A. (symposium editor). *Polyvinylchloride. -2.* (Main lectures presented at the Second International Symposium on Polyvinylchloride, Lyon-Villeurbanne, France, 5-9 July 1976.) Pp.339-567. ISBN-0-08-021203-4. (Oxford and New York: Pergamon Press, 1977.) £13.50.
- HANSON, J. R. (senior reporter). *Terpenoids and Steroids, Vol. 7. (A Review of the Literature Published between September 1975 and August 1976.)* (A Specialist Periodical Report.) Pp.x+349. ISBN-0-85186-316-7. (London: The Chemical Society, 1977. Obtainable from Chemical Society, Blackhorse Road, Leichworth, Herts.: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £25; \$50.
- JOLLY, William L. *The Principles of Inorganic Chemistry.* Pp.viii+376. ISBN-0-07-032758-0. (New York and London: McGraw-Hill Book Company, 1976.) £12.75.
- JONES, J. Bryan, SIH, Charles J., and PERLMAN, D. (edited by). *Applications of Biochemical Systems in Organic Chemistry.* Part 1: Pp.xii+1-506+16. ISBN-0-471-93267-1, Vol. 2: Pp.x+507-1065+16. ISBN-0-471-93270-1. (Techniques of Chemistry, Vol. 10.) (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$73.50; £43.50 the set.
- KERKET, Milton, ZETTEMAYER, Albert C., and ROWELL, Robert L. (edited by). *Colloid and Interface Science, Vol. 1: Plenary and Invited Lectures.* (Proceedings of the International Conference on Colloids and Surfaces: 50th Colloid and Surface Science Symposium, held in San Juan, Puerto Rico on June 21-25, 1976.) Pp.xiii+636. ISBN-0-12-404501-4 (v. 1). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.)
- KOWALSKI, Bruce R. (edited by). *Chemometrics: Theory and Application.* (A symposium sponsored by the Division of Computers in Chemistry at the 172nd Meeting of the American Chemical Society, San Francisco, Calif., Sept. 2, 1976.) Pp.viii+288. ISBN-0-8412-0379-2. (Washington DC: American Chemical Society, 1977.) \$21.
- MANSKE, R. H. F. (edited by). *The Alkaloids: Chemistry and Physiology.* Vol. 16. Pp.xviii+569. ISBN-0-12-469516-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$59; £41.90.
- MILLER, R. Bryan, and WADE, Jr., L. G. (edited by). *Annual Reports in Organic Synthesis-1976.* Pp.xiv+449. ISBN-0-12-040807-4. (New York and San Francisco: Academic Press: a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$16.50; £11.70.
- MITRA, Abhijit. *The Synthesis of Prostaglandins.* Pp.xiii+444. ISBN-0-471-02308-6. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$28.60; £16.90.
- MITTAL, K. L. (edited by). *Micellization, Solubilization, and Microemulsions, Vol. 2.* Pp.xv+945. ISBN-0-306-31024-4(v.2). (New York and London: Plenum Press, 1977.) \$54.
- MOORE, Bradley (edited by). *Chemical and Biochemical Applications of Lasers, Vol. 3.* Pp.ix+325. ISBN-0-12-505403-3(v.3). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$16.50; £11.70.
- MUUS, L. T., ATKINS, P. W., McLAUCHLAN, K. A., and PEDERSEN, J. B. (edited by). *Chemically Induced Magnetic Polarization.* (Proceedings of the NATO Advanced Study Institute held at Sogesta, Urbino, Italy, April 17-30, 1977.) (NATO Advanced Study Institutes Series. Series C: Mathematical and Physical Sciences, Vol. 34.) Pp.xii+407. ISBN-90-277-0845-2. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977. Published in co-operation with Nato Scientific Affairs Division.) Dfl. 95; \$38.
- NOTH, H. (symposium editor). *Boron Chemistry—3.* (Selected lectures presented at the Third International Meeting on Boron Chemistry, Munich and Ettal, FRG., 5-9 July 1976.) (International Union of Pure and Applied Chemistry, Inorganic Chemistry Division, in conjunction with the German Chemical Society.) Pp.691-700. ISBN-0-08-021206-9. (Oxford and New York: Pergamon Press, 1977.) £9.75.
- PEDLEY, J. B., and RYLAND, J. Sussex—N.P.L. *Computer Analysed Thermochemical Data: Organic and Organometallic Compounds.* (Brighton: Dr. J. B. Pedley, School of Molecular Sciences, University of Sussex, 1977. Published with the permission of the Controller of Her Majesty's Stationery Office.) £10.

PROCEEDINGS OF THE SYMPOSIUM ON FLUOROSIS, October 1974. Pp.xvi+534. (Hyderabad, India: Indian Academy of Geoscience, 1977.) Rs.150; \$25; £10.

PULLMAN, Bernard, and GOLDBLUM, Natan (edited by). Excited States in Organic Chemistry and Biochemistry. (Proceedings of the Tenth Jerusalem Symposium on Quantum Chemistry and Biochemistry held in Jerusalem, Israel, March 28/31, 1977.) (The Jerusalem Symposia in Quantum Chemistry and Biochemistry, Vol. 10.) Pp.xiii+448. ISBN-0-277-0853-3. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl. 100; \$39.50.

RASSER, J. C. Platinum-Iridium Reforming Catalysts: TPD of Hydrogen, Selectivity and Activity in Heptane Conversion. Pp.xiii+216. ISBN-90-6275-005-2. (Delft: Delft University Press, 1977.) Dfl. 24.

ROBERTS, M. W., and THOMAS, J. M. (senior reporters). Surface and Defect Properties of Solids, Vol. 6. (A Review of the Recent Literature published up to mid-1976. A Specialist Periodical Report.) Pp.x+367. ISBN-0-85186-300-0. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Leitchworth, Herts: American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D.C.) £27; \$54.

SANDLER, Stanley R., and KARO, Wolf. Polymer Syntheses, Vol. 2. (Organic Chemistry: a Series of Monographs, Vol. 29.) Pp.xi+400. ISBN-0-12-618502-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$39; £28.05.

SCOTT, R. P. W. Liquid Chromatography Detectors. (Journal of Chromatography Library, Vol. 11.) Pp.vii+248. ISBN-0-444-41580-7. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 85; \$34.50.

SEKINE, Tatsuya, and HASEGAWA, Yuko. Solvent Extraction Chemistry: Fundamentals and Applications. Pp.xii+919. ISBN-0-8247-6391-2. (New York and Basel: Marcel Dekker, Inc., 1977.)

SENNING, A., and MAGEE, P. S. (edited by). Topics in Sulfur Chemistry, Vol. 3. Pp. 128. ISBN-3-13-5263-01-0. (Stuttgart: Georg Thieme Publishers, 1977.) DM 58.

SMETS, G. (symposium editor). Photochemical Processes in Polymer Chemistry—2. (Invited lectures presented at the Second IUPAC Symposium, Leuven, Belgium, 2-4 June, 1976.) (International Union of Pure and Applied Chemistry (Macromolecular Division), in conjunction with The University of Leuven.) Pp.403-538. ISBN-0-08-021205-0. (Oxford and New York: Pergamon Press, 1977.) £13.60.

STOCKHAM, John D., and FOCHTMAN, Edward G. (edited by). Particle Size Analysis. Pp.xi+140. ISBN-0-250-40189-4. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$26.40; £15.60.

WEBB, G. A. (edited by). Annual Reports on NMR Spectroscopy, Vol. 7. Pp.xix+300. ISBN-0-12-505307-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £16; \$31.25.

WILLIAMS, John C. (edited by). Preservation of Paper and Textiles of Historic and Artistic Value. (A symposium sponsored by the Cellulose, Paper and Textile Division at the 172nd Meeting of the American Chemical Society, San Francisco, Calif., August 30-31, 1976.) (Advances in Chemistry Series, 164.) Pp.x+403. ISBN-0-8412-0360-1. (Washington, DC: American Chemical Society, 1977.) \$38.

Technology

AMANN, Ronald, COOPER, Julian, and DAVIES, R. W. With the assistance of JENKINS, Hugh. The Technological Level of Soviet Industry. Pp.xxiii+575. ISBN-0-300-02076-7. (New Haven and London: Yale University Press, 1977.) £20.

BARTON, Edwin C., et al. (editorial publication board.) Advances in Automated Analysis: Technicon International Congress, 1976. Vol. 1: Clinical and Hospital Management Symposia. Pp.xxi+490. Vol. 2: Industrial Symposia. Pp.xi+366. (Tarrytown, NY: Mediad, Inc., 1977.)

BOSS '76. Behaviour of Off-Shore Structures. (Proceedings of the First International Conference, held at The Norwegian Institute of Technology, The University of Trondheim, Norway, August 2nd-5th, 1976.) Vol. 1: Pp.1000. ISBN-0-08-021739-7. Vol. 2: Pp.676. ISBN-0-08-022154-8. (Oxford and New York: Pergamon Press, 1977.) £55.50 each volume.

ECKERT, E. R. G., and IRVINE, Jr., Thomas F. (edited by). Progress in Heat and Mass Transfer, Vol. 8: Heat Transfer Reviews 1970-1975. (Monograph Series of the International Journal of Heat and Mass Transfer.) Pp.vii+321. ISBN-0-08-021737-0. (Chicago: Rumford Publishing Company, Oxford and New York: Pergamon Press, 1977.) £19.

GOERGE, Frank. Machine Takeover: The Growing Threat to Human Freedom in a Computer-Controlled Society. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xiii+193. ISBN-0-08-021228-X. (Oxford and New York: Pergamon Press, 1977.) Hard £7.25; Flexi £3.50.

HAHNE, E., and GRIGULL, U. (edited by). Heat Transfer in Boiling. Pp.xiv+486. ISBN-0-12-314450-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers; Washington and London: Hemisphere Publishing Corporation, 1977.) \$39.50; £28.05.

HESKETH, Howard E. Fine Particles in Gaseous Media. Pp.x+214. ISBN-0-250-04182-7. (Ann Arbor, Mich.: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$26.40; £15.60.

HOULDCROFT, P. R. Welding Process Technology. Pp.xix+313. ISBN-0-521-21530-7. (Cambridge, London and New York: Cambridge University Press, 1977.) £8.75 net.

LAUNDER, B. E. (edited by). Studies in Convection. Vol. 2: Theory, Measurement and Applications. Pp.vii+223. ISBN-0-12-438002-6. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £8.80; \$17.25.

NEW INTERNATIONAL DICTIONARY OF REFRIGERATION: Multilingual. Pp.xxvii+560. ISBN-0-08-020368-X. (Paris: Institut International du Froid/International Institute of Refrigeration, 1977. Distributed by Pergamon Press, Oxford.) £43.75.

PAUL, J. K. Solar Heating and Cooling: Recent Advances. Pp.x+485. ISBN-0-8155-0674-0. (Park Ridge, New Jersey: Noyes Data Corporation, 1977.) \$48.

PERRY, Roger, and YOUNG, Robert J. (edited by). Handbook of Air Pollution Analysis. Pp.xiv+506. ISBN-0-412-12660-5. (London: Chapman and Hall, 1977. Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £20.

RECENT ADVANCES IN MINING AND PROCESSING OF LOW-GRADE AND SUBMARGINAL MINERAL DEPOSITS. (Centre for Natural Resources, Energy and Transport, United Nations, New York.) Pp.192. ISBN-0-08-021051-1. (Oxford and New York: Pergamon Press, 1976.) £13.90; \$25.

SCHULTZ, J. M. (edited by). Properties of Solid Polymeric Materials, Part A. (Treatise on Materials Science and Technology, Vol. 10.) Pp.xiv+460. ISBN-0-12-341810-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$44; £31.25.

SHAH, D. O., and SCHECHTER, R. S. (edited by). Improved Oil Recovery by Surfactant and Polymer Flooding. Pp.x+578. ISBN-0-12-641750-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$23.50; £16.70.

SILVERMAN, Joseph, and VAN DYKEN, A. R. (edited by). Radiation Processing. (Transactions of the First International Meeting on Radiation Processing, held at Dorado Beach, Puerto Rico, 9-13 May 1976.) Vol. 1: Invited Papers. Pp.xvii+1402. Vol. 2: Contributed Papers. Pp.xxvii+403-885. ISBN-0-08-021640-4 (2 vol. set). (Oxford and New York: Pergamon Press, 1977.) £55.

TEBBUTT, T. H. Y. Principles of Water Quality Control. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.x+201. ISBN-0-08-021296-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £7.50; Flexicover £3.75.

THOMPSON-RUSSELL, K. C., and EDINGTON, J. W. Electron Microscope Specimen Preparation Techniques in Materials Science. (Philips Technical Library. Monographs in Practical Electron Microscopy in Materials Science, 5.) Pp.136. ISBN-0-333-21980-5. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £9.

YIH, Chia-Shun (edited by). Advances in Applied Mechanics, Vol. 17. Pp.vii+389. ISBN-0-12-002017-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$43; £30.55.

Earth Sciences

BALEK, Jaroslav. Hydrology and Water Resources in Tropical Africa. (Developments in Water Science, 8.) Pp.208. ISBN-0-444-99814-4. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 110; \$44.95.

BARAGAR, W. R. A., COLEMAN, L. C. and HALL, J. M. (edited by). Volcanic Regimes in Canada. (Proceedings of a Symposium held at the University of Waterloo, Waterloo, Ontario, May 16-17, 1975.) (Special Paper No. 16.) Pp.viii+476. (Waterloo, Ontario: Geological Association of Canada, Department of Earth Sciences, The University, 1977.)

CARUTHERS, Jerald W. Fundamentals of Marine Acoustics. (Elsevier Oceanography Series, 18.) Pp.ix+153. ISBN-0-444-41552-5. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 69; \$28.

CHAPMAN, G. P. Human and Environmental Systems: a Geographer's Appraisal. Pp.xiv+421. ISBN-0-12-168650-7. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £12.80; \$25.

DOUGLAS, Ian. Humid Landforms. (An Introduction to Systematic Geomorphology, Vol. 1.) Pp.xvi+288. ISBN-0-262-04054-9. (Cambridge, Mass.: The MIT Press, 1977.) \$15.

GOUDIE, Andrew. Environmental Change. (Contemporary Problems in Geography.) Pp.xi+244. ISBN-0-19-874073-5. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) £7.50 net.

GRANDAL, B., and HOLTET, J. A. (edited by). Dynamical and Chemical Coupling Between the Neutral and Ionized Atmosphere. (Proceedings of the NATO Advanced Study Institute, held at Spatind, Norway, April 12-22, 1977.) (NATO Advanced Institutes Series. Series C: Mathematical and Physical Sciences, Vol. 35.) Pp.xix+392. ISBN-90-277-0840-1. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977. Published in co-operation with NATO Scientific Affairs Division.) Dfl. 90; \$36.

HENIN, S. Cours de Physiques du Col. Tome II: l'eau et le Sol-Les Propriétés Mécaniques-La Chaleur et le Sol. (Initiations-Documentations Techniques, No. 29.) Pp.221. ISBN-2-7099-0417-9. (Paris: ORSTOM; Bruxelles: EDITEC, 1977.) Fr. 45.

HURLBUT, Jr., Cornelius S., and KLEIN, Cornelius. Manual of Mineralogy, 19th Edition (after James D. Dana). Pp.xi+532. ISBN-0-471-42226-6. (New York and London: John Wiley and Sons, 1977.) \$24; £14.

KING, Philip B. The Evolution of North America. Revised edition. Pp.xv+197. ISBN-691-07960-9. (Princeton, NJ: Princeton University Press, 1977.) Cloth £18.70; Paper £7.10.

MCGARY, Noel, and LINCOLN, John H. Tide Prints: Surface Tidal Currents in Puget Sound. (A Washington Sea Grant Publication.) Pp.51. ISBN-0-295-95557-0. (Seattle and London: University of Washington Press, 1977.) £3.75.

MENARD, H. W. (with introductions by). Ocean Science. (Readings from Scientific American.) Pp.307. ISBN-0-7167-0013-1. (San Francisco: W. H. Freeman and Company, 1977.) Cloth \$15; Paper \$8.

MERRILL, R. B. (managing editor). Proceedings of the Seventh Lunar Science Conference, Houston, Texas, March 15-19, 1976. Compiled by The Lunar Science Institute, Houston, Texas. Vol. 1: Regolith Studies. Pp.1-1178. Vol. 2: Petrogenetic Studies of Mare and Highland Rocks. Pp.1179-2542. Vol. 3: The Moon and Other Bodies. Pp.2543-3651. ISBN-0-08-021771-0. (Oxford and New York: Pergamon Press, 1976.) \$160; £90.

MINERALOGY: Towards the Twenty-First Century. (A Discussion organized jointly for The Royal Society and The Mineralogical Society, by J. E. T. Horne and Sir Kingsley Dunham, For. Sec. R. S., in celebration of The Mineralogical Society's Centenary Meeting held on 7 and 8 April 1976.) Pp.ix+4044+27 plates. ISBN-0-86503-092-1. (London: The Royal Society, 1977.) UK £30; Overseas £30.90.

MITCHELL-CHRISTIE, Frank. Practical Weather Forecasting. Pp.96. ISBN-0-86002-097-5. (London: William Luscombe, 1977.) £3.95.

PHILLIPS, O. M. The Dynamics of the Upper Ocean, Second edition. (Cambridge Monographs on Mechanics and Applied Mathematics.) Pp.viii+336. ISBN-0-521-21421-1. (Cambridge, London and New York: Cambridge University Press, 1977.) £16 net.

STATHEM, Ian. Earth Surface Sediment Transport. Pp.viii+184. ISBN-0-19-874076-X. (Contemporary Problems in Geography.) (Oxford: Clarendon Press; London: Oxford University Press, 1977.) Hardcover £6.50; Paperback £3.

TRUDGILL, Stephen T. Soil and Vegetation Systems. (Contemporary Problems in Geography.) Pp.xii+180. ISBN-0-19-874058-1. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) Hardcover £6.50; Paperback £3.

WOLFE, Douglas A. (edited by). Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. (Proceedings of a Symposium, November 10-12, 1976, Olympic Hotel, Seattle, Washington.) Pp.xviii+478. ISBN-0-08-021613-7. (Oxford and New York: Pergamon Press, 1977.)

YOUNG, Louise B. Earth's Aura. Pp.xii+305. (New York: Alfred A. Knopf, 1977.) \$12.95.

Biology

AINSWORTH, Stanley. Steady-State Enzyme Kinetics. Pp.xii+255. ISBN-0-333-15008-2. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £10.

ALBRECHT, Gene H. The Craniofacial Morphology of the Sulawesi Macaques: Multivariate Approaches to Biological Problems. (Contributions to Primatology, Vol. 13.) Pp. viii+152. ISBN-3-8055-2694-6. (Basel, London and New York: S. Karger, 1978.) SFR, DM 74.

AMBROSE, E. J., and EASTY, Dorothy M. Cell Biology. Second edition. Pp.x+556. ISBN-0-17-71033-0. (Sunbury-on-Thames, Middx: Thomas Nelson and Sons, Ltd., 1977.) £5.95 net.

BERTI, F., SAMUELSSON, B., and VELCO, G. P. (edited by). Prostaglandins and Thromboxanes. (NATO Advances Study Institutes Series. Series A Life Sciences, Vol. 13.) Pp.ix+449. ISBN-0-306-35613-9. (New York and London: Plenum Press, 1977. Published in co-operation with NATO Scientific Affairs Division.) \$54.

BIOLOGY AS A SOCIAL WEAPON. Edited by The Ann Arbor Science for the People Editorial Collective, Ann Arbor, Michigan. Pp.vi+153. ISBN-0-8087-4534-4. (Minneapolis, Minnesota: Burgess Publishing Company, 1977.) \$5.95.

BOILMAN, Willi E. Kamuti: a New Way in Bonsai. Pp. 106. ISBN-0-571-11060-6. (London: Faber and Faber, Ltd., 1977. First published 1974.) £1.75 net.

BRIAN, M. V. (edited by). Production Ecology of Ants and Termites. (International Biological Programme, 13.) Pp.xvii+409. ISBN-0-521-21519-6. (Cambridge, London and New York: Cambridge University Press, 1978.) £19.50.

BUXTON, A., and FRASER, G. Animal Microbiology, Vol. 1: Immunology, Bacteriology, Mycology, Diseases of Fish and Laboratory Methods. Pp.viii+357+xxv. ISBN-0-632-00690-0. (Vol. 2: Rickettsias and Viruses. Pp.viii+358-830. ISBN-0-632-00941-1. £25. (Oxford and London: Blackwell Scientific Publications, 1977.)

CARO, C. G., PEDLEY, T. J., SCHROTER, R. C., and SEED, W. A. The Mechanics of the Circulation. (Oxford Medical Publications.) Pp.xiv+527. ISBN-0-19263323-6. (Oxford, New York and Toronto: Oxford University Press, 1978.) £22.

CLARK, Brian F. C. The Genetic Code. (The Institute of Biology's Studies in Biology, No. 83.) Pp.iv+74. ISBN-0-7131-2647-7. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £3.20; Paper £1.60.

COOKE, R. C. Fungi, Man and His Environment. Pp.xiv+144. (London and New York: Longman, 1977.) £6.75.

CORBETT, G. B., and SOUTHERN, H. N. (edited by). The Handbook of British Mammals. Second edition. Pp.xxiii+520. ISBN-0-632-09080-4. (Oxford and London: Blackwell Scientific Publications, 1977. Published for the Mammal Society.) £9.75.

CRAMP, Stanley (chief editor) Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic, Vol. 1: Ostrich to Ducks. Pp.722+108 plates. ISBN-0-19857358-8. (Oxford, London and New York: Oxford University Press, 1977.) £25 net.

DALTON, Stephen. The Miracle of Flight. Pp.168. ISBN-0-562-00070-4. (Maidenhead: Sampson Low, 1977.) £5.95.

DEAN, Roger T. Lysosomes. (The Institute of Biology's Studies in Biology, No. 84.) Pp.52. ISBN-0-7131-2663-9. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £3; Paper £1.50.

EDNEY, E. B. Water Balance in Land Arthropods. (Zoophysiology and Ecology, Vol. 9.) Pp.xii+282. ISBN-3-540-08084-8. (Berlin and New York: Springer-Verlag, 1977.) DM 78; \$34.40.

ELLIS, G. P., and WEST, G. B. (edited by). Progress in Medical Chemistry, Vol. 14. Pp.ix+308. ISBN-0-7204-0645-5. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 110; \$44.95.

ELLORY, J. Clive and LEW, Virgilio L. (edited by). Membrane Transport in Red Cells. Pp.x+470. ISBN-0-12-237150-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £21; \$41.

EVANS, Henry. Botanical Prints, with Excerpts from the Artist's Notebooks. Pp.64. ISBN-0-7167-0192-8. (San Francisco: W. H. Freeman and Company, 1977.) \$25.

FLORKIN, Marcel, NEUBERGER, Albert, and VAN DEENEN, Laurens L. M. (edited by). Comprehensive Biochemistry, Vol. 24: Biological Information Transfer. Pp.xv+301. ISBN-0-444-41583-1. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 82; \$33.50.